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CONTENTS

THE OUTPUT OF THE HEART IN PATIENTS WITH ABNORMAL BLOOD PRESSURES

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Heart failure is preceded by hypertension in a considerable percentage of cases O'Hare, Calhoun and Altnow (1) have recently shown that many patients with heart failure of the type called "chronic myocarditis" or "myocardial insufficiency of unknown origin" have had high blood pressure in the past   In any attempt to analyze the effects of high systemic arterial pressure and to evaluate it as a factor in the causation of heart failure, it is desirable to have knowledge of the other dynamic factors known to be concerned in the work of the heart   The work of the heart is determined chiefly by its output of blood per minute, by the pressure in the pulmonary artery and in the aorta against which it pumps, and by the velocity imparted to the blood by the heart. In the absence of greatly increased output the velocity factor is small relative to the other two, although, as Evans (2) has shown, when large outputs are concerned it may be of major importance

Blumgart and Weiss (3) have studied the velocity of blood flow in patients with arterial hypertension   They found that patients with hypertension who exhibited no evidence of circulatory disability could be divided into two groups in one, the velocity of blood flow was within the limits of normal, in the other the velocity was retarded

Cardiac output in patients with arterial hypertension has been studied by Plesch (4), by Liljestrand and Stenström (5), and by Hayasaka (6)   Plesch's results in four cases showed on the average no definite variation in either direction   Liljestrand and Stenström made repeated observations of the cardiac outputs of five women suffering from increased blood pressure and chronic nephritis   They used the nitrous oxide method of Krogh and Lindhard (7) and found

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that the average cardiac output of these patients was increased 26 per cent above the average of a group of normal subjects   The basal metabolic rate was correspondingly on the average 17 per cent higher than in the control group   The highest outputs were in cases with moderately elevated pressures rather than in those with extremely high ones

The work of Hayasaka, which was done by the triple-extrapolation method of Redfield, Bock, and Meakins (8), gave somewhat confusing results   He used the output per minute per square meter of body surface as the standard of comparison, a value which Burwell and Robinson (9) have shown to be an uncertain one, and found the cardiac output increased in "benign hypertension" and in "malignant hypertension with nephritis," but unchanged in cases with "secondary contracted kidney "   No data concerning variations in metabolic rate are given, but the experiments were performed under standard conditions   The author is of the opinion that hypertension in these cases may be dependent upon the elevated cardiac output, but he also points out that increased blood pressure is not always accompanied by increase in cardiac output

METHODS

The observations here reported were made by the method of Field, Bock, Gildea and Lathrop (10) with careful attention to the constancy of "standard basal" conditions as described in previous publications (11)   In each case, observations were made only after several days of rest in hospital had led to the establishment of a relatively fixed level of bood pressure   Whenever possible, more than one observation was made on each subject   To emphasize the ease with which this method may be applied to patients untrained in respiratory methods and as evidence for the reliability of the results obtained there is presented in table 1 a record of all the differences in arterial and venous carbon dioxide tension observed in these experiments   It will be noted that in each experiment the arterio-venous differences agree satisactorily, usually within 1 mm and that the maximum variation is 1 7 mm

Five patients with arterial hypertension were studied, three men and two women   The systolic pressures ranged between 175 mm and

225 mm mercury and the diastolic between 110 mm and 140 mm Although we attempted to select relatively uncomplicated cases all of the patients, as may be seen from the appended clinical summaries, showed evidence of more or less arteriosclerosis and several of them had undoubted chronic nephritis One patient (case 5) had had a partial thyroidectomy four months before our study was made, and at the time of observation had a slightly elevated basal metabolic rate No patient had evidence of congestive heart failure at the time of observation and only one (case 3) had a history suggesting its presence previously All patients in the group showed definite evidence of cardiac enlargement in the teleoroentgenogram or by per-

TABLE 1

Arterio-venous differences in carbon dioxide tension (millimeters of mercury)

Subject number	1	2	2	3	3	4	4	5	5	6	7	7	8	9
Experiment number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	11 1	7 8	6 3	8 1	9 8	9 9	8 2	8 6	7 4	7 6	7 8	6 8	8 0	11 9
2	10 3	7 4	6 9	7 9	9 8	8 4	9 9	8 4	7 3	7 3	7 7	8 3	8 0	11 7
3	11 5	6 8	7 1	8 7	8 9	9 2	9 3	8 7	7 2	7 5	7 2	7 1	7 9	10 9
4				7 5	9 6	8 6	8 6	8 3	7 4	7 4				11 5
5						8 8	9 0	8 3						
Average	10 9	7 3	6 8	8 1	9 5	8 9	9 0	8 5	7 3	7 5	7 6	7 4	8 0	11 4

cussion In four subjects a second estimation of cardiac output was made after an interval of one or more days

Similar observations were carried out upon four men exhibiting systolic blood pressures of from 65 mm to 95 mm of mercury and diastolic pressures ranging from 55 mm to 70 mm Of these, one was a case of rapidly progressive Addison's disease, in which the diagnosis was subsequently confirmed by autopsy To within 48 hours of our observation he had been receiving frequent injections of adrenalin and also whole dried adrenal gland by mouth Another subject was a young man suffering from tuberculosis of the lungs and cecum and probably Addison's disease Two measurements were made of this patient's cardiac output. Of the two other subjects, Cases 8 and 9, one was a member of the hospital staff and the other a medical student,

both apparently quite normal, but having low systolic blood pressure under the imposed conditions of fasting and rest

For convenience in comparison certain average values are calculated In order to avoid distortion because some patients have two and some only one set of figures, the averages are obtained by calculating first the average values for each subject and then computing the average of these figures

RESULTS

The results of the studies are given in table 2   The cardiac output of all the subjects in both groups falls within the limits seen in normal healthy people when the blood pressures are within the usual zone The averages of the cardiac outputs in the two groups are almost identical and the average cardiac output per minute per kilogram of body weight and the average output of the heart per beat are of the same general order   The absence of variation is striking when it is observed that the average pulse pressure in the hypertensive group is 85 mm as against 26 mm in the hypotensive group

The basal metabolic rate is on the whole somewhat higher in the group with high pressures than in the group with low   This is partly explicable on the score of the elevated rate of subjects 4 and 5, on the one hand, and the characteristically low rate of the patient with Addison's disease, on the other   The cardiac output per 100 cc oxygen absorbed is, however, of the same order in the two groups The pulse rates in both groups with the exception of those of the two normal men are slightly above the low level usually attained under satisfactory "basal" conditions, and the average rate is higher in the hypertensive cases, just as is the metabolic rate   The arterio-venous differences do not differ significantly in the two groups

DISCUSSION

These observations demonstrate that under conditions of bodily rest patients with arterial hypertension have no significant increase in the cardiac output   The hypothesis that hypertension in general is secondary to an increased output is thus not tenable   An admissible objection to this conclusion is the possibility that the cardiac output was increased earlier in the history of these patients but that at the

TABLE 2

The cardiac output and related data in patients with high and low blood pressures

Date	Case number	Experiment number	Systolic (mm. mercury)	Diastolic (mm. mercury)	Pulse pressure (mm. mercury)	Vital capacity Actual (cc.)	Vital capacity Per cent normal	Carbon dioxide per min. (cc.)	Arterio-venous difference (vol. per cent)	Cardiac output per minute (cc.)	Cardiac output per kilo per minute (cc.)	Cardiac output per 100 cc. oxygen (cc.)	Cardiac output per beat (cc.)	Pulse rate (per min.)	Respiratory quotient	Basal metabolic rate (per cent)	Weight in kilos
February 13 1928	1	1	215	125	90	3,700	71	195	4 58	4,250	58	1,740	51	84	0 80	+3	73
February 18 1928	2	2	205	110	95	2,100	75	118	3 12	3,780	88	2,380	49	77	0 74	−7	43
February 20 1928	3	3	200	110	90	2,100	75	126	2 90	4 350	101	2,560	59	74	0 76	−4	43
April 12, 1928		4	187	130	57	3 000	72	176	3 34	5,270	83	2 150	68	78	0 72	+2	63 5
April 16 1928	4	5	172	120	50	2 600	61	164	3 96	4,140	66	1,960	54	76	0 78	−11	63 5
April 29 1928		6	225	140	85	3,400	91	199	3 70	5,380	96	2,270	63	85	0 84	+20	56
April 30 1928	5	7	225	140	85	4 000	107	196	3 74	5,240	94	2,160	62	85	0 81	+22	50
June 20, 1928		8	175	120	55	2,500	91	173	3 63	4 770	95	2,120	44	108	0 77	+25	50
June 22, 1928		9	190	120	70	2 500	91	174	3 13	5,560	111	2 530	58	96	0 79	+22	50
Average in hypertensive patients					85		71		3 65	4 700	85	2,160	56	84		+7	57 1
December 7, 1927	6	10	65	45	20	4,300	98	166	3 78	4,390	70	1 960	52	84	0 83	−17	63
February 24, 1928	7	11	85	55	30	3 300	88	164	3 27	5 040	114	2,230	55	92	0 72	+8	44
March 3, 1928		12	80	55	35	3,200	85	158	3 17	5 000	114	2 270	57	88	0 72	+4	44
March 18, 1928	8	13	82	65	17	4 100	86	178	3 15	5,650	83	2,600	84	66	0 82	−15	68
April 14, 1928	9	14	95	60	35	5 000	100	198	5 01	3,950	47	1 650	62	64	0 83	−13	84
Average in hypotensive patients					26				3 75	4 750	75	2,075	64	76		−10	65

time of observation they had reached a stage of the disease when the heart could no longer maintain so great an increase   Against the possibility of this it may be said that these were patients without signs or symptoms of heart failure and capable without distress of degrees of exertion certainly demanding greater cardiac output

These observations show also that the increase in the work of the heart leading to its hypertrophy in patients with actual hypertension is due not to changes in velocity or cardiac output but to increase in resistance, unless exercise in hypertensive patients is accompanied by a disproportionate increase in output   Holman and Beck (12) from a comparison of the heart weights in two similar dogs, one of which had pulmonic stenosis and the other a large septal defect, infer that increased flow of blood through the heart is a more potent stimulus to hypertrophy and dilatation than increased peripheral resistance The stimulus in our cases, however, was apparently increased resistance since no elevation of the cardiac output was observed

A comparison of the hypertensive and hypotensive groups shows a striking lack of relationship between blood pressure and cardiac output   Here are patients with very high and very low systolic pressures, and with great variations in pulse pressure, but with nearly identical cardiac outputs   Blumgart and Weiss (3) pointed out the fact that hypertension does not necessarily imply any change in the velocity of blood flow   If increase in tension were necessarily followed by increase in cardiac output, or by increased velocity, the work of the heart would be still further increased to its further hurt   These observations must not be thought to show that hypertension and greatly increased cardiac output are never associated   They often are, as with the development of severe anemia in chronic nephritis with hypertension   And hypotension may, for example, be associated with distinctly diminished cardiac output in animals in shock as Blalock (13) has shown   The association of hypertension and increased output, or hypotension and decreased output, is accordingly not a necessary one

These studies bear on the question of proportionality between pulse pressure and output per beat, a relation suggested by Erlanger and Hooker (14), who were fully aware of its limitations   Rosen and White (15) have recently brought evidence to show that the pulse

pressure is directly proportional to the output per beat in consecutive observations in the same individual under conditions that produce essentially the same diastolic pressure and heart rate. Our experiments show a complete lack of proportionality in different individuals, a lack of correspondence due, as Wiggers (16) points out, to variations in vascular elasticity and tonicity, the viscosity of the blood, and other factors

SUMMARY

In two groups of cases, one with systolic blood pressures of over 175 mm of mercury and one with systolic blood pressures of less than 95 mm of mercury, the total cardiac output per minute, the cardiac output per minute per kilogram of body weight and the cardiac output per 100 cc of oxygen absorbed did not show significant differences Pulse pressure and output per beat are not directly proportional in different individuals

PROTOCOLS

1 F H , a white male age 59 years, complained of headache, dizziness, impaired vision, weakness and impairment of memory for 5 years He was known to have had an elevated blood pressure for 5 years, and nocturia seven to eight times for 1 year On physical examination he was a plethoric individual, with advanced arterio-sclerosis and vascular retinitis. The cardiac dulness was 11 5 cm to the left and 4 cm to the right The heart sounds were of good quality, no murmurs were heard There was no pitting edema or other evidence of cardiac failure There were albuminuria and cylindruria but no nitrogen retention.
The diagnosis was hypertension, arterio-sclerosis, chronic nephritis

2. E T , a colored woman of 55 years, had palpitation of the heart, weakness, and dyspnea on exertion for five years There had been some precordial pain during one year Examination showed a small, fairly well nourished colored woman The eyegrounds showed vascular retinitis with retinal hemorrhages The cardiac dulness was 9 0 cm to the left and 4 0 cm. to the right and the supra cardiac dulness was 5 8 cm wide. There were no evidences of cardiac failure, no anemia, and no evidence of nephritis
The diagnosis was arterio-sclerosis, hypertension

3 E C , a colored male aged 36 years complained of frequent attacks of pal pitation of the heart for 14 months and some nocturnal dyspnea for 10 years

He had a primary syphilitic lesion 17 years ago   On physical examination he was well nourished and developed   Examination of the eyegrounds showed an early arteriosclerosis   The teleoroentgenogram of the heart showed it to be 10 3 cm to the left, 3 7 cm to the right, and the aortic shadow was 6 4 cm in diameter The heart sounds were of good quality, there was a soft systolic murmur at the apex but no evidence of cardiac failure   There was very little peripheral sclerosis and no evidence of renal disease   The blood Wasserman was positive

The diagnosis was hypertension, early vascular disease, syphilis

4 A J , a white male, aged 41 years, was known to have had hypertension and chronic nephritis for two and a half years   Headache was the most persistant and severe symptom   Examination showed a small fairly well nourished man with no evidence of cardiac failure but with marked peripheral arterial sclerosis and eyeground changes   A seven foot plate of the heart showed it to be 8 5 cm to the left, 3 6 cm to the right, and the aortic shadow was 6 5 cm   The heart sounds were of poor quality, but there were no murmurs   The blood contained 41 mgm of non-protein nitrogen per 100 cc   there was constant albuminuria, and the specific gravity of the urine was low and fixed   There was no anemia

The diagnosis was chronic nephritis, hypertension, and arteriosclerosis

5 F B , a white female of 26 years, admitted with a history of thyrotoxicosis four months previous to this entry   Thyroidectomy was performed at this time, this was followed by improvement but the blood pressure, which was known to have been elevated for seven months, remained high   She developed palpitation of the heart and slight dyspnea on exertion   On physical examination she was rather poorly nourished but there were no definite signs of thyrotoxicosis   The cardiac dulness was 9 5 cm to the left   There was very little peripheral sclerosis, no evidence of renal impairment and no edema or other evidence of cardiac failure The basal metabolic rates on successive days were 25 and 22 per cent above the calculated normal

The diagnosis was hypertension, thyrotoxicosis (mild), early vascular disease

6 F B , a white male aged 40 years, complained of weakness and shortness of breath for one month previous to admission   The blood pressure was known to have been low for one month   On examination he was fairly well nourished and developed   There was some increased pigmentation of the exposed parts of the skin.   There was no anemia   The cardiac dulness was not increased, the sounds were of poor quality, but there was no evidence of congestive heart failure   Adrenalin and extract of the whole gland produced no improvement and the patient died twenty days after admission   Post-mortem examination showed extreme atrophy of the adrenals

The diagnosis was Addison's disease

7 C B , a white male aged 24 years, complained of abdominal cramps and weakness for one year, with a loss of 36 pounds in 8 months.   On examination the patient was found to be emaciated and rather pale with pigmentation suggesting Addison's disease.   The cardiac dulness was 3 cm. to the right, 6 cm to the left and the supra-cardiac dulness was 4 cm   There were a few moist râles in both lung apices. There was some tenderness and resistance in the lower right quadrant of the abdomen   The red blood cells numbered 4,510,000 per cm.   The hemoglobin was 63 per cent.

The diagnosis was bilateral pulmonary tuberculosis, tuberculosis of the cecum, and Addison's disease (?)

8 W G , was a normal, healthy medical student 23 years of age.

9 M H., was a member of the hospital staff, 29 years of age and quite well

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THE EFFECT OF THE DIGITALIS BODIES ON THE VELOCITY OF BLOOD FLOW THROUGH THE LUNGS AND ON OTHER ASPECTS OF THE CIRCULATION A STUDY OF NORMAL SUBJECTS AND PATIENTS WITH CARDIOVASCULAR DISEASE[1]

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Knowledge of the efficiency of the circulation in man before and after the administration of therapeutic measures is essential to establish a rational basis for the treatment of circulatory failure Circulatory measurements in normal subjects and in patients before and after the administration of digitalis bodies are few Burwell, Neighbors, and Regen (1) studied the changes in the minute volume output of the heart after the administration of large therapeutic doses of digitalis powder to four normal subjects Following the administration of digitalis they observed, by the method of Field, Bock, Gildea, and Lathrop (2), no increase in the cardiac output per minute On the contrary there was a slight but definite reduction of blood flow The decrease in blood flow, according to these authors, could not be explained by the normal variation in the volume of blood expelled from the left ventricle per minute, or by technical error In applying their results to patients with circulatory failure, they caution, however, that "If the same pharmacological effect upon heart muscle can produce different effects upon cardiac output in different states of heart muscle, it is then unsafe to apply our conclusions directly to an analysis of the effect of digitalis upon the cardiac output of patients suffering from heart failure" Eppinger, Papp, and Schwarz (3) observed a reduction in the amount of blood expelled from the left

[1] This investigation was aided by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases

ventricle of one patient who was suffering from circulatory failure No significance can be attached to this finding for it is well recognized that the method they used does not give reliable results in patients with circulatory failure Cohn and Stewart (4) studied the effect of digitalis by means of moving x-ray films, observing the change in the height of the left ventricular excursion of the heart The four patients studied were young adults with symptoms and signs of rather mild circulatory failure Two of the patients showed auricular fibrillation, the other two regular rhythm In all four patients a significant increase in the height of the left ventricular excursion was seen, when the patients were under the effect of digitalis An increase in the ventricular excursion, under such conditions, does not necessarily indicate, but is suggestive of increased output of the heart, provided that there is no corresponding decrease in rate, and provided there is no significant change in the size of the heart Recently Hochrein and Meier (5), applying Bromser's method on man, noted that, following the administration of strophanthin, patients suffering from circulatory failure showed a decrease between the peaks of the pulse and pulse velocity curves simultaneously taken This indicates that, following the administration of therapeutic doses of strophanthin, the blood flow through the radial arteries increased Kinnmonth (6) using Henderson's ethyl iodid method observed that the circulation rate may be increased, decreased, or unaffected by digitalis in patients with diseased circulatory system These variations in reponse to giving digitalis occurred not only in different patients, but in the same patient at different times An increase in the circulation rate was more apt to occur in patients with low circulatory rate and with signs of circulatory failure In patients with heart disease, but with no reduction of the circulatory rate, an increase occurred less frequently and the circulation rate was either unaltered or decreased It should be recalled that the value of the ethyl iodid method in the form applied by Kinnmonth has been adversely criticised

In view of these discrepant results, and the questionable reliability of the methods applied by some of the investigators, one is forced to conclude that the effect of digitalis on the blood flow in man and in patients with circulatory failure remains unknown

Because preceding studies (7) have shown that the velocity of blood flow is a fundamental and characteristic aspect of the circulation both in health and in disease, and because a reliable method for the measurement of the velocity of blood flow has become available, we have investigated the effect of digitalis on the velocity of blood flow in normal subjects as well as in patients with cardiovascular disease

METHODS

The methods employed in this investigation were similar to those used in previous studies (8)   Simultaneously with the measurement of the velocity of the blood flow through the lungs, the velocity of venous blood flow from the right elbow to the right auricle was measured   The venous pressure was estimated by the method of Moritz and Tabora (9), and the vital capacity of the lungs with a Collins spirometer   All measurements were performed after more than 12 hours of eating the last meal

RESULTS

A In normal subjects

To ascertain the effect of the digitalis bodies on the velocity of blood flow of normal human beings, eight volunteer male subjects between the ages of 24 and 46 were studied (table 1)   To observe the immediate effect of the administration of digitalis bodies, four of the subjects received strophanthin intravenously   The physiological activity of the specimen used was 0 14 mgm per cat unit, as estimated by the assay of Hatcher and Brody (10)   The velocity of the blood flow and other aspects of the circulation were measured before and again after the administration of the strophanthin   In subjects 4, 6, and 8 the measurement of velocity was repeated three hours after injection of the drug, while in subjects 3 and 5 the tests were repeated six hours after the administration of strophanthin   Subjects 1, 6, and 7 received large therapeutic doses of tincture of digitalis by mouth   The physiological potency of the tincture used corresponded to 1 cat unit per 1 cc   Subjects 1 and 6 received an initial dose of 50 per cent of the total dose at once, followed by two doses

TABLE 1

Effect of digitals on the velocity of pulmonary blood flow of normal subjects

Number	Name	Age		Date	Heart rate per minute	Arm to heart circulation time		Pulmonary circulation time		Medication	Method of administration
						Seconds	Change, seconds	Seconds	Change, seconds		
				1927							
1	V F	46	Before	March 16	70	6 5	+3 5	14 0	+3 5	Tr dig 19 cc	Mouth
			After	March 17	58	10 0		17 5			
2	W H	35	Before	January 19	103	5 5	+0 5	8 0	+0 5	Stroph 0 9 mgm	Vein
			After	January 19	91	6 0		8 5			
3	J M	35	Before	January 18	83	5 0	+0 5	8 0	0 0	Stroph 0 7 mgm	Vein
			After	January 18	93	5 5		8 0			
4	J D	24	Before	January 18	79	7 5	+4 5	11 5	0 0	Stroph 0 9 mgm	Vein
			After	January 19	83	12 0		11 5			
5	C W	35	Before	January 19	112	6 0	+2 0	8 5	−0 5	Stroph 0 7 mgm	Vein
			After	January 19	97	8 0		8 0			
6	I D	41	Before	January 16	55	13 0	+4 5	12 0	−1 0	Tr dig 17 cc	Mouth
			After	January 17	57	8 5		11 0			
7	L I	21	Before	January 6	59	5 0	+0 5	8 5	−2 5	Tr dig 20 cc	Mouth
			After	January 18	54	5 5		11 0			
8	M M	35	Before	January 19	111	5 0	−1 0	10 0	−2 5	Stroph 0 9 mgm	Vein
			After	January 19	100	4 0		7 5			

of 25 per cent of the total calculated dose at six hour intervals   The circulatory tests were performed six hours after the last dose   Subject 7 received tincture of digitalis in the same manner as just described   Circulatory measurements in the case of this individual were performed 12 hours after the administration of the last dose, when this normal person showed electrocardiographic evidence of the effect of digitalis   The same measurements were repeated twelve days later, when the dose of digitalis previously administered had probably been eliminated   Toxic reactions except for nausea and vomiting in subjects 1 and 7 were not observed   Table 1 illustrates that although there was a variation in dosage and method of administration of the digitalis bodies, the velocity of the blood flow showed no significant alteration   The average difference between the circulation time of the venous blood flow from the cubital vein to the right side of the heart was an increase of 1 9 seconds, the average difference between the pulmonary circulation times of the eight normal subjects studied was a decrease of 0 3 second   The maximum variation before and after the administration of the digitalis bodies was an increase of 4 5 seconds in the venous circulation time, and an increase of 3 5 seconds in the pulmonary circulation time. We have called attention to the fact that the velocity of the venous blood flow from the arm to the right side of the heart shows greater spontaneous variations both in normal individuals and in patients with circulatory failure, than the velocity of the blood flow in the pulmonary circuit (8)   The arterial and venous blood pressures, as well as the vital capacities of the lungs of the eight subjects studied, showed no changes which could be attributed to a digitalis effect

B In patients with cardiovascular disease

The results of the investigation of the effect of the digitalis bodies on the velocity of blood flow of patients suffering from cardiovascular disease is presented in table 2   A summary of the clinical observations concerning the cardiovascular system of the 14 patients is appended at the end of this publication   It is evident that both the abnormality of the cardiovascular system and the degree of circulatory failure varied considerably   Patients 7, 8, 9, 10, 11 and 14 exhibited only symptoms and signs of circulatory failure on exertion,

TABLE 2

Effect of digitalis bodies on the velocity of pulmonary of blood flow of patients suffering from cardiovascular diseases

Number	Name	Age	Diagnosis		Date	Pulse	Venous pressure cm. H₂O	Vital capacity per square meter cc	Arm to heart circulation time Seconds	Change, seconds	Pulmonary circulation time Seconds	Change, seconds	Medication	Method of administration
1	J M	62	Myocardial degeneration	Before	March 6	100	+9 0	800	22 0	−6 0	45 0	−13 0	Tr dig 16 cc.	Mouth
				After	March 8	72	+6 5	900	16 0		32 0			
2	J C	57	Myocardial degeneration	Before	February 23	84	+5 5	1,361	13 0	0 0	16 5	−8 0	Tr dig 22 cc	Mouth
				After	February 23	87	+4 0	1,951	13 0		8 5			
3	P F	63	Arterial hypertension, myocardial degeneration	Before	May 12	81	+4 5	1,790	8 0	0 0	21 0	−7 0	Tr dig 16 cc	Mouth
				After	May 14	73	+2 0	1,790	8 0		14 0			
4	C B	41	Arterial hypertension, myocardial degeneration	Before	May 18	118	+21 0	650	22 0	−15 5	22 0	−6 0	Tr dig 20 cc	Mouth
				After	May 21	82	+6 5	1,960	6 5		16 0			
5	T J	52	Generalized arteriosclerosis, myocardial degeneration	Before	February 15	74	+4 5	1,960	10 0	+2 0	22 0	−5 0	Tr dig 18 cc	Mouth
				After	February 17	68	+2 0	1,960	12 0		17 0			
6	P M	50	Myocardial degeneration	Before	May 12	64	+3 5	964	11 0	+4 0	14 5	−4 5	Tr dig 21 cc	Mouth
				After	May 16	58	−1 0	1,384	15 0		10 0			
7	A S	74	Arterial hypertension, myocardial degeneration	Before	March 6	78	+5 0	2,330	9 0	+5 0	24 5	−4 5	Tr dig 16 cc	Mouth
				After	March 8	66	+2 0	2,610	14 0		20 0			

8	P P	68	Auricular fibrillation, arterio sclerosis	Before After	February 13 February 15	88 75	+5 5 +5 5	2,230 2,230	10 0 8 0	−2 0	15 5 12 0	−3 5	Stroph 1 0 mgm.	Ven
9	W M	65	Syphilitic heart disease, aortic insufficiency	Before After	March 8 March 10	72 74	+2 0 +2 5	2,000 1,950	10 0 7 0	−3 0	17 0 16 0	−1 0	Tr dig 19 cc.	Mouth
10	A. O	68	Arterial hypertension, myocardial degeneration	Before After	January 6 January 6	78 60	+7 0 +10 5	1,790 1,440	10 0 8 5	−1 5	13 0 15 0	+2 0	Stroph. 0 8 mgm	Ven
11	W P	39	Syphilitic heart disease, aortic insufficiency	Before After	March 8 March 10	84 72	+1 0 +1 0	2,130 2,090	5 0 5 5	+0 5	12 0 14 0	+2 0	Tr dig 17 cc	Mouth
12	D K	61	Myocardial degeneration, auricular fibrillation	Before After After	February 21 February 23 February 28	80 64 60	+4 0	580 1 580 1,790	14 0 15 0 14 0	+1 0 0 0	20 0 25 0 21 0	+5 0 +1 0	Tr dig 20 cc	Mouth
13	L S	39	Rheumatic heart disease, mitral stenosis and insufficiency	Before After	March 16 March 17	62 55	−5 0 −4 5	2,730 2,730	13 5 10 0	−3 5	15 0 22 0	+7 0	Tr dig. 14 cc.	Mouth
14	S C	26	Rheumatic heart disease, pan carditis with auricular fibrillation	Before After	December 7 December 7	76 62	+10 0 +8 5	1,690 1,590	9 5 15 5	+6 0	19 0 26 0	+7 0	Stroph 0 8 mgm.	Ven

while patients 1, 2, 3, 4, 5, 6, 12 and 13 showed signs of congestive failure of the circulation even when at rest   Patients 8, 12 and 14 exhibited fibrillation of the auricles, while in the other patients the rhythm was regular   In order to differentiate between the effect of rest and of digitalis, all patients were kept in bed for from 8 to 30 days, until clinical observations, electrocardiographic measurements, and tests of the vital capacity indicated no further improvement Tested tincture of digitalis or strophanthin was then administered in large therapeutic doses   The effects of full digitalization were evidenced within 24 hours after beginning the administration of the drug   Circulatory measurements were then repeated within 24 hours after the administration of the last dose in all cases with the exception of patient 6, in whom the measurements were repeated 2 days after the last dose   All patients showed one or more effects of digitalization, such as slowing of the pulse rate, inversion of the T waves in the leads of the electrocardiographic tracings, or the appearance of nausea   Several patients showed several of these effects As the main interest in this study was to observe the effect of the digitalis bodies on the pulmonary blood flow, which is closely related to the state of the entire body, the data for the patients are grouped in table 2 according to the degree of change in the pulmonary circulation rate following the administration of tincture of digitalis or strophanthin   Patient 1 showed the most marked decrease, and patient 14 the most marked increase of the pulmonary circulation time   The results indicate that only three patients (12, 13, and 14) of the fourteen patients studied showed a definite slowing of the pulmonary blood flow   In one of these three patients (number 12) six days after the beginning of the administration of digitalis the velocity was the same as before   In the other eleven patients the velocity of pulmonary blood flow was either unaltered or there was a definite increase as the result of the administration of the digitalis bodies   Accepting 3 5 seconds as the maximum normal variation, as was shown by the measurements on normal subjects, seven of the fourteen patients showed a definite increase above normal   The average increase in these seven patients was 6 9 seconds   The findings indicate no relationship between the changes in the velocity of pulmonary blood flow and that of the venous blood flow from the arm

to the heart   The vital capacities of the lungs showed no definite changes after the administration of digitalis   The venous pressure became definitely lowered in patients 1 and 4   No relationship could be established between the severity of circulatory failure and changes in velocity   The pulse rate was lower in all the patients with the exception of patients 2 and 9   The average slowing of the cardiac rate was 12 per minute (from 81 beats to 69)   Definite correlation could not be established between the degree of circulatory failure and change in the pulmonary circulation time, although six of the seven patients who reacted with a definite increase in velocity showed symptoms and signs of congestive failure at the time of test   These patients were numbers 1, 2, 3, 4, 5 and 6   It is of interest to note that two of the three patients who exhibited fibrillation of the auricles showed definite prolongation of the pulmonary circulation time after the administration of large therapeutic doses of tincture of digitalis   These were patients 12 and 14   Patient 8, however, showed a decrease of 3 5 seconds

<p align="center">DISCUSSION</p>

The results of this study indicate that tincture of digitalis and strophanthin, and thus presumably other digitalis bodies, when administered to normal subjects under the conditions described, exert no appreciable change on the velocity of the pulmonary blood flow   When digitalis and strophanthin were administered to patients with cardiovascular disease the velocity of flow in the pulmonary circuit became distinctly slower in three patients, while in the rest of the eleven patients there was either no change, or a definite increase   The observations also indicate that following the administration of digitalis changes are greater in cardiac patients than in normal subjects

Our observations in the past (7) (11) demonstrated that in circulatory failure there is slowing of the velocity of blood flow   Although slowing is not always proportional to the degree of clinical decompensation, with clinical improvement there is nevertheless generally an increase in the velocity of blood flow   The cardiac output of the heart in circulatory failure is not definitely known at present because of technical difficulties connected with the measurements   The conception expressed by certain investigators that with circulatory failure

there is an increase in the cardiac output could be reconciled with the findings of slowing of the pulmonary velocity only if one assumes that in cardiac failure there is considerable increase in the available cross sectional area of the vascular bed   At present experimental or clinical data are not available which throw light on this aspect of the problem   Similarly, no direct reliable observation on patients with circulatory failure is available which supports the theory that the beneficial effect of digitalis on the circulation manifests itself in a sedative action, decreasing the minute volume output of the heart   Our finding that seven out of fourteen patients with circulatory failure showed an increase in the velocity of blood flow in the pulmonary circuit following the administration of digitalis, is in harmony with other numerous observations on patients, in whom during their stay in hospital repeated measurements of the velocity of blood flow were taken   According to these observations, with improvement in the general clinical condition of the patient, the velocity of blood flow and the vital capacity increased and the venous pressure became lower

The change in velocity of the blood flow following the administration of digitalis is therefore the same as the change which follows other therapeutic measures, which are beneficial in the treatment of circulatory failure   The observation that those patients who showed clinical improvement after the administration of digitalis bodies showed likewise an increase in the velocity of blood flow is of greater significance in the analysis of digitalis effect in patients, than finding no change or slowing   To condemn the use of digitalis because it fails to improve the circulation in a few patients is as irrational as giving up arsenicals because they do not always better patients with syphilis   It is often observed clinically that, depending probably on structural and physiological changes in the heart, a stage is reached when digitalis fails to benefit   In rare instances the symptoms and signs of circulatory failure may become definitely worse after the administration of digitalis and improvement may follow the omission of the drug   Our observations suggest that the digitalis bodies do not induce uniform changes in the velocity of blood flow of patients suffering from circulatory failure   This is in harmony with the findings of others on the effect of digitalis on the minute volume output of the heart, as well as on the cardiac rate, conducting

system, and vital capacity   If we consider that the degree as well as the nature of pathological changes in the structures through which digitalis influences the circulation vary markedly from patient to patient, it is questionable whether a uniform change in circulatory measurements after the administration of digitalis bodies can be expected

The fact that seven patients showed definite increase in the velocity of pulmonary blood flow indicates that the digitalis bodies are useful agents in the treatment of circulatory failure   Since the relatively direct approach to the problem of the action of digitalis in patients with circulatory failure entails a number of variables it can be readily understood how hazardous is the application of observations gained in studying animals, or normal men

SUMMARY AND CONCLUSIONS

1 Strophanthin and tincture of digitalis were administered intravenously and by mouth respectively to eight normal persons   Their effect on the velocity of pulmonary and peripheral venous blood flow, on the vital capacities of the lungs and arterial and venous pressures, were observed   Amounts of these drugs corresponding to large therapeutic doses failed to change appreciably the velocity of the pulmonary blood flow and the other above mentioned aspects of the circulation in the normal subjects

2 When strophanthin, or tincture of digitalis in large therapeutic doses was administered to 14 patients suffering from cardio vascular disease, the velocity of the pulmonary blood flow became increased in seven, was unaltered in four, while in three patients it was definitely decreased

3 Although the average pulse rate in seven patients showed a reduction of 14 beats per minute, the pulmonary circulation time showed an average reduction of 6 9 seconds, which corresponds to an increase of 30 per cent in the velocity of pulmonary blood flow

4 The velocity of blood flow in the pulmonary circuit is decreased in patients with circulatory failure   With clinical signs of improvement due to the administration of digitalis or to rest the velocity of this blood flow increases, although the degree of the patient's improvement and the change in velocity may not be parallel

5 Patients with symptoms and signs of congestive failure, even when at rest, have had a greater tendency to show definite increase in the velocity of blood flow in the pulmonary circuit following the administration of digitalis than patients who were compensated at rest

6 The studies presented help to provide a rational basis for the therapeutic administration of digitalis to patients suffering from circulatory failure

ABSTRACTS OF HISTORIES AND PHYSICAL EXAMINATIONS OF PATIENTS STUDIED

1 J M , aged 62, complained of increasing dyspnea of one and a half years' duration   For three months he had been unable to walk because of marked weakness   He noticed marked swelling of the abdomen and legs   He was cyanotic and dyspneic   The heart was moderately enlarged   The sounds were distant   The cardiac rate per minute was 130 to 140   The rhythm was regular   The blood pressure was 120 mm systolic and 50 mm diastolic of mercury   Over the base of the lungs moist bubbling râles were heard   The abdomen was distended   The liver edge was felt 5 cm below the costal margin   Marked pitting edema was noted over the buttocks and legs   The condition of the patient was unaltered at the time of the administration of digitalis   The diagnosis of myocardial degeneration was made

2 J C , aged 57, complained of shortness of breath and inability to work for one and a half years   He noticed swelling of the feet following exertion   The heart was normal in size   The cardiac rate was rapid   The sounds were distant   No murmurs were heard   At time of the administration of the digitalis bodies the patient was slightly dyspneic   The diagnosis of myocardial degeneration was made

3 P F , aged 63, was suffering from nocturnal attacks of dyspnea   During the last two years he had become weak and unable to work   Occasionally he noticed swelling of the ankles   On physical examination the heart was enlarged, the greatest diameter being 14 cm , and the aortic second sound was markedly accentuated   The rhythm was regular   Slight pitting edema was noticed over the ankles   The blood pressure was 210 mm of systolic and 120 mm diastolic of mercury   The patient's condition was unchanged at the time of the administration of digitalis   The diagnosis of arterial hypertension was made

4 C B , aged 41, complained of shortness of breath and weakness for one year   He noticed gradually increasing swelling of the ankles and legs during the six months previous to his entry to hospital   He was unable to walk during the same period   On physical examination he showed marked dyspnea and orthopnea   The lips were cyanotic   The heart was large   The greatest transverse diameter was 13 cm   The heart sounds were normal, and no murmur was heard   The

rate was rapid, 110 to 130 The rhythm was regular There was marked swelling of the abdomen with a suggestive fluid wave The liver area was tender The legs were swollen with marked pitting edema. The blood pressure was 195 mm systolic and 160 mm diastolic of mercury The patient's condition did not improve under rest in bed, and the symptoms and signs of circulatory failure were unchanged at the time of administration of digitalis The diagnosis of arterial hypertension was made

5 Th J, aged 52, had been well until fourteen months previous to his entrance into the hospital, when he noticed cramp like pain starting in the precordium and radiating to the epigastrium This attack was repeated several times, he became very dyspneic on such occasions During the last four weeks he noticed marked swelling of the ankles, which disappeared after rest During the last two weeks he also coughed a great deal and has had several attacks of palpitation The apex impulse was observed in the sixth interspace. The first sound at apex was short and merged into a soft systolic murmur The second sound was reduplicated all over the precordium The cardiac rhythm was regular The peripheral vessels were sclerosed A moderate degree of edema was observed over both ankles at the time of entrance into the hospital The blood pressure was 140 mm systolic and 100 mm diastolic of mercury At the time of test the general condition of the patient was the same, except that no edema was observed The diagnosis of arteriosclerotic heart disease was made.

6 P M, aged 50, noticed one month previous to his entry increasing shortness of breath, cough, swelling of the legs and abdomen He was unable to work On physical examination he was markedly orthopneic The apex of the heart was not felt The heart sounds were distant. The cardiac rate was rapid and the rhythm regular The blood pressure was 155 mm systolic and 80 mm diastolic of mercury The right side of the chest posteriorly was flat on percussion and the breath sounds were suppressed Moist bubbling râles were heard over the base on both sides The abdomen was tender over the liver area and it was large. The legs were markedly swollen The condition was essentially unchanged at the time of administration of digitalis The diagnosis of myocardial degeneration was made

7 A S, aged 74, complained of frequent attacks of precordial pain, associated with dyspnea No other evidence of circulatory failure was obtained from the history On physical examination some tortuosity and sclerosis of the retinal vessels were observed The lips were cyanotic The heart was of normal size. The heart sounds were weak but regular The peripheral vessels were markedly thickened. The blood pressure was 190 mm systolic and 95 mm diastolic of mercury Numerous moist râles were heard over the bases of the lungs. The diagnosis of arteriosclerotic heart disease and arterial hypertension was made

8 P P, aged 68, complained of shortness of breath and weakness He had noticed swelling of the feet frequently after work On physical examination the heart was found enlarged The maximum of the transverse diameter was 13 cm.

The sounds were rather distant   The rhythm was totally irregular   The peripheral vessels were moderately sclerosed   The blood pressure was 120 mm systolic and 70 mm diastolic of mercury   No edema was noted   The condition of the patient at the time of test was essentially unchanged   The diagnosis of arteriosclerosis and auricular fibrillation was made

9 W M , aged 65, had had shortness of breath during the last six months previous to his admission   Two weeks previous to entry he noticed sharp abdominal pains which became aggravated after meals   He never observed swelling of the ankles, or of the abdomen   Physical examination showed an enlarged heart with a maximum transverse diameter of 14 cm   The heart sounds were of good quality and the pulmonary second sound was accentuated   Over the third right intercostal space a rough systolic murmur and a prolonged blowing diastolic murmur were heard   The peripheral vessels were moderately thickened   The pulses were full and bounding   Blood pressure was 196 mm systolic and 50 mm diastolic of mercury   No pitting edema or other signs of congestive cardiac failure observed   The Wassermann test of the blood was positive   The diagnosis of syphilitic heart disease with aortic insufficiency was made

10 A O , aged 68, gave no history of circulatory failure   He had been told that his blood pressure was high   The heart was slightly enlarged   The greatest transverse diameter was 12 cm on percussion   The aortic second sound was markedly accentuated   The blood pressure was 228 mm systolic and 112 mm of diastolic   The diagnosis of arterial hypertension was made

11 W P , aged 39, suffered from attacks of precordial pain of two years' duration   During the last three weeks he had been suffering from shortness of breath which was worse at night   He also coughed a great deal during the same period   He had had no edema of the ankles despite the fact that he walked much   Physical examination showed marked pulsation of the vessels of the neck, over which a systolic murmur was heard   The apex impulse of the heart was felt in the sixth intercostal space in the anterior axillary line   The heart was enlarged downward and outward   The sounds were of good quality, with regular rhythm   Loud to and fro murmur was heard over the precordium with the maximum intensity over the aortic area   The radial pulses were of the Corrigan type   The blood pressure was 164 mm systolic and 40 mm diastolic of mercury   The Wassermann and Kahn tests of the blood were positive   The patient's condition was the same as just described at the time of the test   The diagnosis of syphilitic heart disease with aortic insufficiency was made

12 D K , aged 61, had had shortness of breath on exertion for one year   One month previous to his admission he became so weak that he could walk short distances only   No history of edema was obtained   The complaints were essentially the same as at the time of test   On physical examination the cardiac impulse was rather diffuse   The maximum transverse diameter was 13 cm   The sounds were of fairly good quality   At the apex a soft systolic murmur was heard   The rhythm was totally irregular   The peripheral vessels showed

moderate thickening   The blood pressure was 110 mm systolic and 70 mm. diastolic of mercury   The liver edge was 3 cm below the costal border   The diagnosis of arteriosclerotic heart disease and auricular fibrillation was made

13 L S , aged 39, was suffering from severe attacks of rheumatic fever at the age of 18   For one year he had noticed shortness of breath on exertion, as well as occasional swelling of the ankles   He was slightly dyspneic. The heart was slightly enlarged, the greatest transverse diameter being 12 cm   The first sound over the apex was loud   Over the same area a systolic and diastolic murmur was heard   The rhythm was regular   The liver was not palpated. Slight pitting edema over the ankles was noted   The blood pressure was 108 mm systolic and 60 mm diastolic of mercury   Under rest the patient improved and at the time of administration of digitalis he was not dyspneic and no edema was present   The diagnosis of rheumatic heart disease with mitral stenosis and insufficiency was made

14 S C , aged 26, complained of shortness of breath   He had had rheumatic fever in childhood but had been well until nine years previously, when after pneumonia, he developed moderate shortness of breath for eight months   During the ten months before entry he experienced slight precordial pain on exertion with shortness of breath and palpitation, which gradually increased in severity forcing him to enter the hospital. Three days before admission, pitting edema was observed over lower legs   After admission to the hospital, on rest in bed and on taking digitalis, he showed moderate improvement   Physical examination showed blowing systolic and diastolic murmurs over aortic and mitral areas   The liver was not palpable   There was no edema of the legs   A few moist rales were heard over the left base   Following rest in hospital patient returned home but was compelled to re-enter hospital because of exacerbation of symptoms   At time of administration of strophanthin patient was up and about the ward   There was no dyspnea or orthopnea. Physical examination showed no rales over chest and no edema   The liver was not palpable, and the heart was as noted above   The diagnosis was rheumatic pericarditis, auricular fibrillation, aortic stenosis and insufficiency, mitral stenosis and insufficiency

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OBSERVATIONS ON PAROXYSMAL HEMOGLOBINURIA[1]

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WITH THE TECHNICAL ASSISTANCE OF EMILY FRÜHBAUER

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the Mary Imogene Bassett Hospital, Cooperstown, N Y)

For the discovery of the reaction which reproduces in vitro what
appears to be the essential feature of the mechanism of this disease we
are indebted to Donath and Landsteiner (1)   They showed that the
serum of these patients contains an auto-hemolysin which unites with
the patient's own red blood cells or with those of any other individual
only at a low temperature, that when such a mixture is warmed to
37°C hemolysis occurs if complement be present   Complement is
essential for the completion of the reaction   Rosenbach (2) had pre-
viously shown that all the phenomena of a typical paroxysm of the
disease may be produced by immersion of the hands or feet of the
patient in ice water

During the past nine years I have had five of these patients under
observation and this paper summarizes some of the serological, clinical
and therapeutic results

This auto-hemolysin has certain striking peculiarities   At body
temperature it will not unite with the red blood cells   The highest
temperatures at which union could be detected with the auto-hemoly-
sins from three of the patients I have studied were respectively 12°C ,
12°C and 10°C (protocol 1)   Temperatures down to 4°C or 5°C ,
however, gave more complete union, shown by greater hemolysis
when the mixture was subsequently warmed   In two of these patients
hemolysis was greater if the red cells from the patient were used rather
than those from another individual of the same blood group (pro-
tocol 2)   In all five of these patients it has been possible to confirm
the observation of Yorke and Macfie (3) that union is more effective

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and hemolysis greater if the chilling is limited to five or ten minutes rather than the more usual period of thirty minutes (protocol 3) This is particularly interesting in view of what occurs when a patient spontaneously has a paroxysm   The actual time during which the

PROTOCOL 1

December 7, 1922   Patient L O   Blood drawn from arm vein, one portion allowed to clot at room temperature, serum separated 30 minutes after venepuncture, serum in ice box over night.   A second portion was taken in citrate and used for the preparation of the red cell suspension   Control serum and red cell suspension prepared in the same way from a normal adult belonging to the same blood group

Tube	Patient's serum unheated	Patient's red blood cells 5 per cent	Control serum unheated (Group A)	Control red blood cells 5 per cent (Group A)	Complement 1 10 (guinea pig)	NaCl 0 85 per cent	Kept at	Hemolysis
	cc	cc	cc	cc	cc	cc		
1	0 25	0 1			0 1	0 05	0°C 10 minutes, 37°C 2 hours	++
2	0 25			0 1	0 1	0 05		++
3	0 25	0 1			0 1	0 05	4°C 10 minutes, 37°C 2 hours	+(+)
4	0 25			0 1	0 1	0 05		++
5	0 25	0 1			0 1	0 05	8°C 10 minutes, 37°C 2 hours	+(+)
6	0 25			0 1	0 1	0 05		+(+)
7	0 25	0 1			0 1	0 05	12°C 10 minutes, 37°C 2 hours	+
8	0 25			0 1	0 1	0 05		+
9	0 25	0 1			0 1	0 05	16°C 10 minutes, 37°C 2 hours	0
10	0 25			0 1	0 1	0 05		0
11			0 25	0 1	0 1	0 05	0°C 10 minutes, 37°C 2 hours	0
12		0 1	0 25		0 1	0 05	0°C 10 minutes, 37°C 2 hours	0
13	0 25	0 1			0 1	0 05	Not chilled, 37°C 2 hours	0
14			0 25	0 1	0 1	0 05	Not chilled, 37°C 2 hours	0
15				0 1	0 1	0 3	0°C 10 minutes, 37°C 2 hours	0
16		0 1			0 1	0 3	0°C 10 minutes, 37°C 2 hours	0

blood in superficial capillaries is exposed to lowered temperatures is obviously short   It soon passes back to the higher temperatures of the interior of the body

These auto-hemolysins also have in some instances a surprising thermolability   In one case 45°C for thirty minutes destroyed the auto-hemolysin so that the addition of fresh complement would not reacti-

vate it, in another case it was destroyed at 47 5°C The extreme lability of this strange hemolysin is further shown by the fact that sometimes after one to three days in the ice box the serum will no longer cause lysis of the red cells The hemolysin has disappeared (protocol 6) It has been shown repeatedly that the serum of par-

PROTOCOL 2

January 2, 1925   Patient F V   Blood drawn from arm vein one portion was allowed to clot at room temperature, serum separated as soon as clot had formed and test set up at once a second portion was citrated and used for the preparation of the red cell suspension   Control serum and red cell suspension obtained from a normal adult belonging to the same blood group as the patient.

Tube	Patient s serum un heated	Patient s red blood cells 5 per cent	Control serum unheated (Group O)	Control red blood cells 5 per cent (Group O)	Complement 1 10 (guinea pig)	NaCl 0 85 per cent	Kept at	Hemolysis
	cc.	cc	cc.	cc	cc	cc		
1	0 3	0 1			0 1		0°C 10 minutes, 37°C. 2 hours	++
2	0 25	0 1			0 1	0 05	0°C. 10 minutes, 37°C. 2 hours	+(+)
3	0 2	0 1			0 1	0 1	0°C 10 minutes, 37°C 2 hours	+
4	0 1	0 1			0 1	0 2	0°C 10 minutes, 37 C 2 hours	0
5	0 3			0 1	0 1		0°C. 10 minutes, 37°C 2 hours	+
6	0 25			0 1	0 1	0 05	0 C. 10 minutes, 37°C. 2 hours	0
7	0 2			0 1	0 1	0 1	0 C. 10 minutes, 37°C 2 hours	0
8	0 1			0 1	0 1	0 2	0°C 10 minutes, 37 C 2 hours	0
9		0 1	0 25		0 1	0 05	0 C 10 minutes, 37°C. 2 hours	0
10			0 25	0 1	0 1	0 05	0°C 10 minutes 37 C. 2 hours	0
11		0 1			0 1	0 3	0°C 10 minutes, 37°C 2 hours	0
12				0 1	0 1	0 3	0 C 10 minutes, 37°C 2 hours	0
13	0 3	0 1			0 1		Not chilled, 37°C 2 hours	0
14	0 25	0 1			0 1	0 05	Not chilled, 37°C. 2 hours	0

oxysmal hemoglobinurics causes lysis not only of the patient's own red cells but also of the red cells of any other individual   I have made several attempts to separate by absorption experiments the auto-hemolysin from the iso-hemolysin (protocol 4)   But in no case was one removed without also removing the other, hence they are probably identical

Whether it is necessary to have complement present at the low temp-

erature is a question which has been answered in different ways by different observers (4, 5) So far as my observations go they indicate that complement may be added after the chilled serum and cells have been warmed to 37 5° and still have hemolysis occur, but that if alexin be present in the mixture at the low temperature, more complete hemolysis occurs (protocol 5)

PROTOCOL 3

November 29, 1921 Patient L O Blood drawn from arm vein, one portion allowed to clot at room temperature, serum separated as soon as coagulation was complete and test set up immediately, a second portion was citrated and used for the preparation of the red cell suspension Control serum and red cell suspension obtained in the same way from a normal adult of the same blood group

Tube	Patient's serum unheated	Patient's red blood cells 5 per cent	Control serum unheated (Group O)	Control red blood cells 5 per cent (Group O)	Complement 1 10 (guinea pig)	NaCl 0 85 per cent	Kept at	Hemolysis
	cc.	cc	cc	cc	cc	cc		
1	0 25	0 1			0 1	0 05	0°C 5 minutes, 37°C 2 hours	+++
2	0 25	0 1			0 1	0 05	0°C 7 minutes, 37°C 2 hours	+++
3	0 25	0 1			0 1	0 05	0°C 10 minutes, 37°C 2 hours	+++
4	0 25	0 1			0 1	0 05	0°C 15 minutes, 37°C 2 hours	++
5	0 25	0 1			0 1	0 05	0°C 20 minutes, 37°C 2 hours	++
6	0 25	0 1			0 1	0 05	0°C 30 minutes, 37°C 2 hours	+(+)
7		0 1	0 25		0 1	0 05	0°C 10 minutes, 37°C 2 hours	0
8			0 25	0 1	0 1	0 05	0°C 10 minutes, 37°C 2 hours	0
9				0 1	0 1	0 3	0°C 10 minutes, 37°C 2 hours	0
10		0 1			0 1	0 3	0°C 10 minutes, 37°C 2 hours	0
11	0 25			0 1	0 1	0 05	0°C 30 minutes, 37°C 2 hours	++(+)
12	0 25	0 1			0 1	0 05	Not chilled, 37°C 2 hours	0
13	0 25			0 1	0 1	0 05	Not chilled, 37°C 2 hours	0

Another question upon which discordant results have been reported (6, 7) is the possible effect of CO_2 either as a substitute for chilling or as an auxiliary factor in bringing about union of the hemolysin and the red cells The experiments I have done with CO_2 were entirely negative (8) No effect upon union nor upon hemolysis was observed

That there is probably some undefined factor in the mechanism of the disease appears to be indicated by observations on two of our

December 5, 1921   Patient L. O   Blood drawn from arm vein one portion allowed to clot at room temperature serum separated as soon as coagulation was complete and the experiment set up immediately, a second portion was citrated and used for the preparation of the red cell suspension   Control serum and red cell suspension obtained in the same way from a normal adult of the same blood group

2.0 cc. of the patient's serum was mixed with 2 0 cc. of washed and packed red blood cells of the patient and immersed in melting ice for 15 minutes.   The mixture was then centrifuged cold and the supernatant serum quickly pipetted off   This is "Patient's serum 'auto'-absorbed

The same absorption of the patient's serum in the cold was carried out with an equal volume of washed and packed red blood cells from a normal individual of the same blood group   This is "Patient's serum 'iso'-absorbed."

Tube	Patient's serum "auto" absorbed unheated	Patient's serum "iso" absorbed unheated	Patient's serum untreated unheated	Patient's red blood cells 5 per cent	Control red blood cells 5 per cent (Group A)	Control serum unheated (Group A)	Complement 1 10 (guinea pig)	NaCl 0.85 per cent	Kept at	Hemolysis
	cc	cc	cc	cc	cc	cc.	cc	cc.		
1	0 25			0 1			0 1	0 05	0°C 10 minutes, 37°C. 2 hours	0
2	0 25				0 1		0 1	0 05	0°C 10 minutes, 37°C. 2 hours	0
3		0 25		0 1			0 1	0 05	0°C. 10 minutes, 37 C. 2 hours	0
4		0 25			0 1		0 1	0 05	0 C. 10 minutes, 37°C 2 hours	0
5			0 25	0 1			0 1	0 05	0°C. 10 minutes, 37°C. 2 hours	++++
6			0 25		0 1		0 1	0 05	0°C 10 minutes 37°C 2 hours	++++
7					0 1		0 1	0 3	0°C. 10 minutes, 37°C. 2 hours	0
8					0 1		0 1	0 3	0°C. 10 minutes, 37°C. 2 hours	0
9				0 1		0 25	0 1	0 05	0°C 10 minutes, 37°C. 2 hours	0
10					0 1	0 25	0 1	0 05	0°C. 10 minutes, 37 C. 2 hours	0
11	0 25			0 1			0 1	0 05	Not chilled, 37 C. 2 hours	0
12	0 25				0 1		0 1	0 05	Not chilled, 37°C 2 hours	0
13		0 25		0 1			0 1	0 05	Not chilled, 37°C. 2 hours	0
14		0 25			0 1		0 1	0 05	Not chilled, 37°C. 2 hours	0

Titration of the Wasserman reaction on the "auto"-absorbed and the untreated serum used in the above experiment gave the following results

	0 02 cc.	0.01 cc.	0 006 cc.	0.003 cc.	0 001 cc.	0.0005 cc.
Untreated serum						
Alcoholic antigen	++++	++++	++++	++++	+	0
Cholesterin antigen	++++	++++	++++	++++	+	0
"Auto"-absorbed serum, not inactivated						
Alcoholic antigen	++++	++++	++++	++++	+	0
Cholesterin antigen	++++	++++	++++	++++	++	±
"Auto"-absorbed serum, inactivated						
Alcoholic antigen	++++	++++	++++	++++	++	0
Cholesterin antigen	++++	++++	++++	++++	+	0

patients   One was a boy of 7 and the other a boy of 8   One had an extreme susceptibility to spontaneous attacks and a low titer of hemolysin in his serum and plasma, the other had paroxysms only after relatively long exposure to low temperatures and a high titer of hemolysin in his serum and plasma   There seems to be no explanation for such an inverse relationship between the titer of hemolysin and the susceptibility to attacks unless one postulates some factor as yet undemonstrated   The fact that the boy with a low titer of hemolysin and high susceptibility to attacks was subject to very marked vaso-motor disturbances while the other boy had none that were apparent,

PROTOCOL 5

November 11, 1920   Patient J B   Serums, red cell suspensions and complement prepared in the usual way   One portion of the patient's serum was inactivated at 55°C for 30 minutes

Tube	Patient's serum un heated	Patient's serum in activated	Patient's red blood cells 5 per cent	Complement 1 10 (guinea pig)	NaCl 0 85 per cent	Kept at	Hemolysis
	cc	cc	cc	cc	cc		
1	0 25		0 1	0 1	0 05	0°C 30 minutes, 37°C 2 hours	+++
2		0 25	0 1		0 05	0°C 30 minutes, 37°C 2 hours	0
3		0 25	0 1	0 1	0 05	0°C 30 minutes, 37°C 2 hours	++
4		0 25	0 1		0 05	0°C 30 minutes Tube warmed to 37°C then 0 1 cc complement added Kept at 37°C 2 hours	+

All the usual controls were negative

would suggest that a vasomotor mechanism may have something to do with the hemolysis

From the etiological and from the therapeutic point of view the most important aspect of this disease is its relation to syphilis   Many of the reported cases have been in congenital syphilitics   The Wasserman reaction is positive in nearly every case and in many of the adult cases late manifestations of acquired syphilis have been present (9, 10)   It was of interest therefore to attempt the separation of the auto-hemolysin from the Wassermann reacting substance   This has

January 27, 1925   Patient G C   Blood drawn from arm vein, divided into two portions serum and red cell suspension prepared in the usual way   Test set up immediately

Tube	Patient s serum un- heated	Patient s red blood cells 5 per cent	Comple- ment 1 10 (guinea pig)	NaCl 0 85 per cent	Kept at	Hemolysis
	cc	cc	cc	cc		
1	0 3	0 1	0 1		0°C. 10 minutes, 37 C. 2 hours	++++
2	0 25	0 1	0 1	0 05	0°C. 10 minutes, 37°C 2 hours	++++
3	0 2	0 1	0 1	0 1	0°C. 10 minutes, 37°C. 2 hours	++++
4	0 15	0 1	0 1	0 15	0°C. 10 minutes, 37 C 2 hours	+++
5	0 10	0 1	0 1	0 2	0°C. 10 minutes, 37 C. 2 hours	+++
6	0 05	0 1	0 1	0 25	0°C. 10 minutes, 37°C. 2 hours	+
7	0 01	0 1	0 1	0 29	0°C. 10 minutes, 37°C 2 hours	±

The usual controls were negative

Titration of the Wassermann reaction on the same serum

	0 1 cc.	0 02 cc	0 01 cc.	0 006 cc.	0 003 cc.	0 001 cc.
Alcoholic antigen	++++	++++	++++	++++	++++	+++
Cholesterin antigen	++++	++++	++++	++++	+++	++

The serum was then left in the ice box for 3 days.   Repetition of the above titrations January 30 1925

Tube	Patient's serum un heated	Patient's red blood cells 5 per cent	Com- plement 1 10 (guinea pig)	NaCl 0 85 per cent	Kept at	Hemolysis
	cc.	cc	cc.	cc.		
1	0 3	0 1	0 1		0°C. 10 minutes, 37°C 2 hours	0
2	0 25	0 1	0 1	0 05	0°C. 10 minutes, 37°C 2 hours	0
3	0 2	0 1	0 1	0 1	0°C. 10 minutes, 37°C. 2 hours	0
4	0 15	0 1	0 1	0 15	0 C. 10 minutes, 37°C 2 hours	0
5	0 10	0 1	0 1	0 2	0°C. 10 minutes, 37°C 2 hours	0
6	0 05	0 1	0 1	0 25	0°C. 10 minutes, 37°C. 2 hours	0
7	0 01	0 1	0 1	0 29	0°C. 10 minutes, 37 C. 2 hours	0

The usual controls were negative

Titration of the Wassermann reaction on the same serum

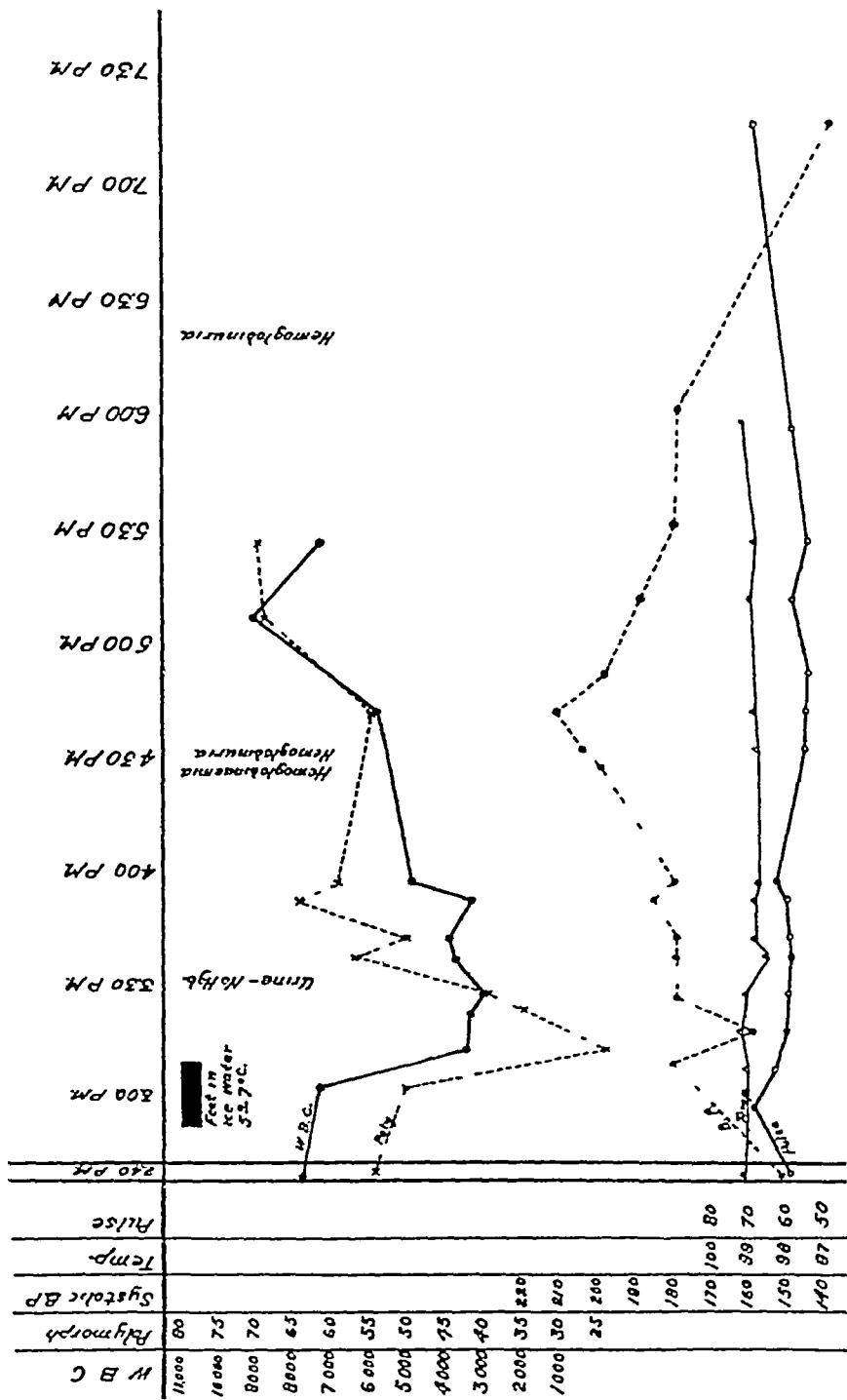|	0 1 cc.	0 02 cc.	0 01 cc.	0 006 cc.	0 003 cc.	0 001 cc.
Alcoholic antigen	++++	++++	++++	++++	+++	++
Cholesterin antigen	++++	++++	++++	++++	+++	+

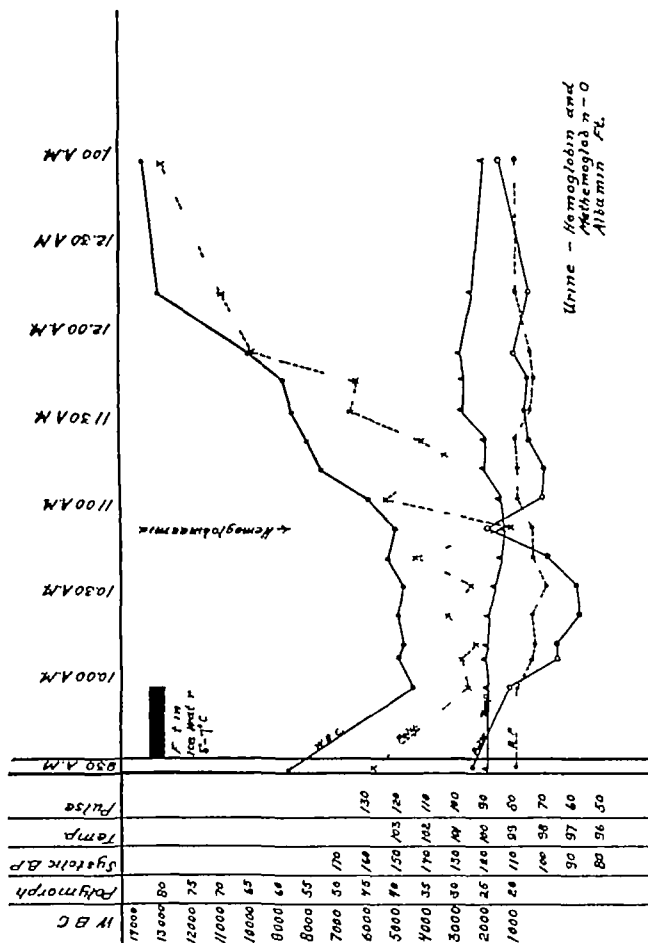CHART 1 PATIENT J R (APRIL 27, 1922) ARTIFICIALLY INDUCED ATTACK OF HEMOGLOBINURIA

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CHART 2 PATIENT J B (JULY 11, 1923) ARTIFICIALLY INDUCED HEMOGLOBINEMIA AND ALBUMINURIA. NO HEMO
GLOBINURIA EXAMPLE OF A LARVAL ATTACK

been done in two ways   The auto-hemolysin was completely absorbed out of hemoglobinuric serum and the Wassermann reaction titrated before and after the absorption of the hemolysin (protocol, 4)   Such an experiment shows quite regularly that the auto-hemolysin may be

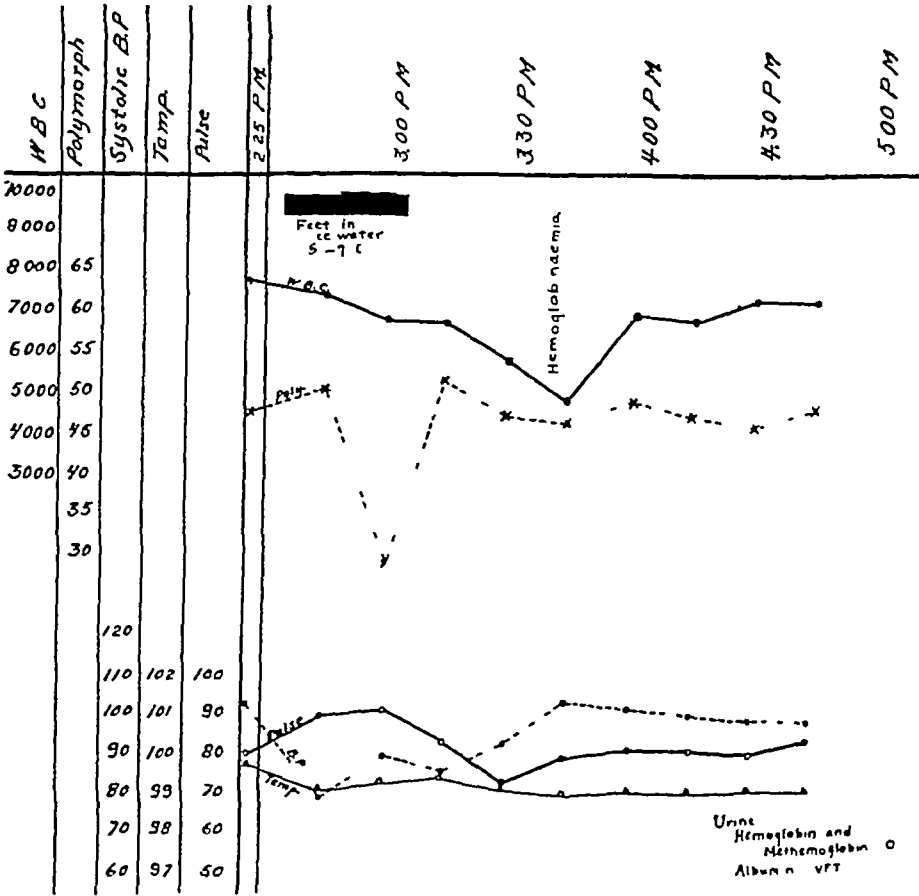CHART 3   PATIENT G C   (DECEMBER 20, 1926)   ARTIFICIALLY INDUCED HEMOGLOBINEMIA AND ALBUMINURIA   NO HEMOGLOBINURIA EXAMPLE OF A LARVAL ATTACK

completely removed without perceptibly weakening the Wassermann reaction   The same result is obtained if hemoglobinuric serum is allowed to stand in the ice-box until the auto-hemolysin has disappeared (protocol 6)   It will be found that the Wassermann reacting
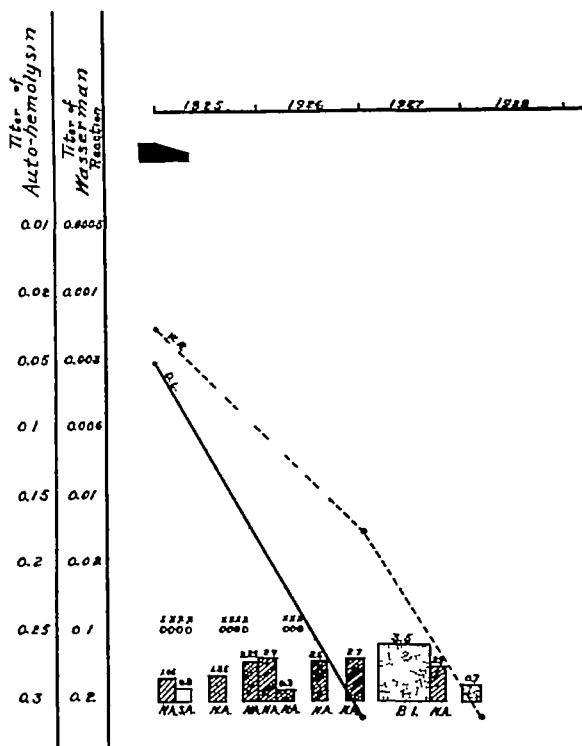
CHART 4   EFFECT OF ANTISYPHILITIC TREATMENT ON AUTO-HEMOLYSIN, W R.
AND SYMPTOMS   PATIENT G C

The period during which spontaneous attacks of hemoglobinuria occurred is
shown in black at the top of the chart. At the bottom of the chart the kind and
amount of treatment is indicated A = arsphenamine NA = neoarsphenamine
SA = sulpharsphenamine BI = intramuscular and intravenous injections of
bismuth   The amount of each of these drugs given is shown in grams at the top
of the columns representing the different drugs $\times \times \times$ = inunctions of mer
cury or intramuscular injections $\bigcirc \bigcirc \bigcirc$ = potassium iodide. Line WR =
Wassermann reaction   Line DL = Donath Landsteiner reaction

substance shows no significant change   Furthermore, comparison
of the titers of the two reactions in a series of patients shows that the
titers do not parallel each other   Such results were perhaps to be

CHART 5   EFFECT OF ANTISYPHILITIC TREATMENT UPON AUTO-HEMOLYSIN, W R
AND SYMPTOMS   PATIENT L O
Symbols as in Chart 4

expected from the dissimilarity of the effects of temperature upon
the two substances [2]

[2] Burmeister reported experiments in which he found that absorption of par-
oxysmal hemoglobinuria serum by erythrocytes removed both the auto hemolysin
and the Wassermann reacting substance   In view of the opposite results of Moro
and Noda (12), Matsuo (10) and Kaznelson (13) and those reported in this paper,
Burmeister's conclusions should not be accepted without confirmation

Certain very striking vasomotor phenomena are often observed during paroxysms of this disease (14)   Two of the five patients had Raynaud's syndrome during attacks, one of these was found to have a systolic blood pressure of 250 mm Hg during a spontaneous paroxysm His usual systolic pressure was 140 to 150 mm Hg
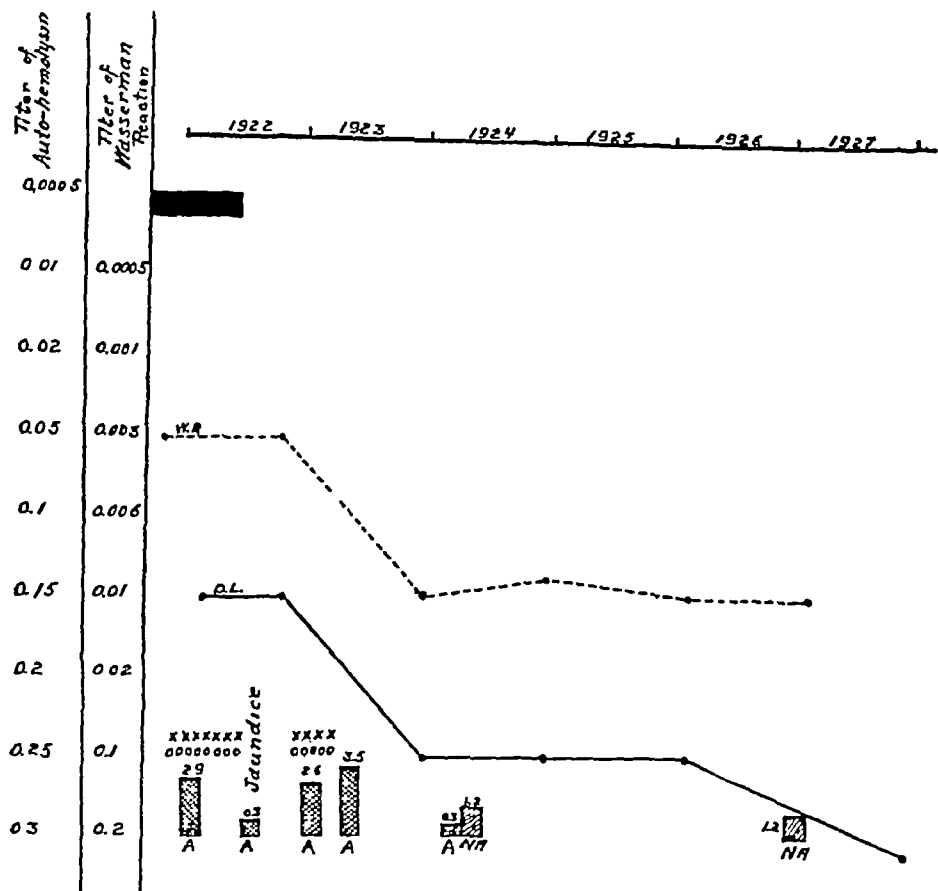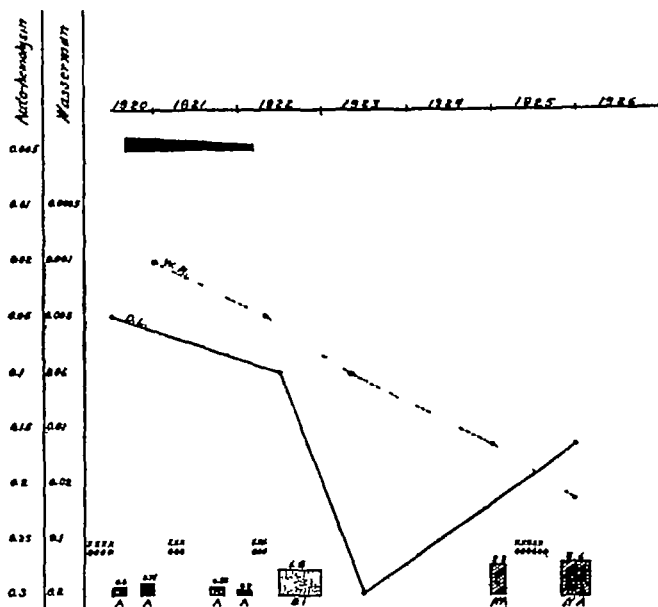

CHART 6 EFFECT OF ANTISYPHILITIC TREATMENT UPON AUTO-HEMOLYSIN, W R
AND SYMPTOMS   PATIENT J B
Symbols as in Chart 4

When a paroxysm of hemoglobinuria is artificially induced (Rosenbach test) by immersion of the hands or feet in ice water there may be, in addition to hemoglobinemia and hemoglobinuria, changes in blood pressure, temperature and the leucocyte count, the crise hemoclasique of Widal   Chart 1 shows the results of such an experiment   The

TABLE 1

Summary of clinical, serological and therapeutic observations on 5 patients with paroxysmal hemoglobinuria

Case	Duration	Seasonal occurrence	History relating attacks to chilling	Wassermann reaction	Clinical evidence of syphilis	Vasomotor phenomena	Susceptibility to attacks	Donath and Landsteiner Result	Donath and Landsteiner Titer	Rosenbach test	Hemoclastic reaction	Kind of treatment	Result of treatment	Blood group	Remarks
J B (F) Age 9	4½ years	Fall, winter, spring	+	++++	Hutchinson teeth, enlarged liver	None	++	+	0 05	±	+	Anti luetic	Cessation of attacks, Donath and Landsteiner and Wassermann reaction still positive	A	,
I O (M) Negro Age 58	1 year	Winter	+	++++	Enlarged tender and irregular liver	None	++	+	0 15	+	±	Irregular anti luetic	Cessation of attacks Donath and Landsteiner and Wassermann reaction still positive	A	Donath and Landsteiner later negative Wassermann reaction still positive
J R (M) Negro Age 10	1½ years	Fall, winter, spring	Indefinite	++++	None	Raynaud's syndrome	+++	+	0 07	+	+	Very irregular anti luetic	No effect	O	Died lobar pneumonia Type III 1926
C C (M) Negro Age 8	6 weeks	Winter	+	++++	Hutchinson teeth ?	None	+++	+	0 003	±	+	Intensive anti luetic	Prompt cessation of attacks Later Donath and Landsteiner and Wassermann reaction negative	AB	
F V (M) Age 7	4 years	All the year, more in winter	+	++++	Saddle nose Hutchinson teeth, large liver and spleen, anemia	Hives, Raynaud's syndrome	++++	+	0 2	Not done	Not done	Irregular anti luetic	General condition improved Attacks continued Donath and Landsteiner and Wassermann reaction positive	O	

total white count and the percentage of polymorphonuclears show an abrupt drop after the feet have been in ice water for a few minutes Synchronous with the decrease in polymorphonuclear cells there is an increase in the percentage of lymphocytes. After one to two hours the leucocytes return to normal, and this may be followed by a leucocytosis due to an increase above normal of the polymorphonuclear cells (chart 2). The blood pressure may be unaffected or may show a gradual increase over a period of about two hours following the chilling (chart 1). Charts 2 and 3 are examples of larval attacks artificially induced. In these experiments hemoglobinemia occurred but there was no hemoglobinuria. Nevertheless, the characteristic changes in the blood picture were observed

The five patients on whom observations have been made were all given anti syphilitic treatment (15). Two of these patients did not return with sufficient regularity to receive adequate treatment. The results of treatment on the other three patients are shown in charts 4, 5, and 6. It is perhaps noteworthy that the patient (G C) receiving the most intensive treatment showed the most prompt cessation of symptoms and the earliest disappearance of the Donath and Landsteiner and the Wassermann reactions, even though the Donath and Landsteiner had a higher titer in this patient than in any other patient of the series

Table 1 summarizes some of the clinical, serological and therapeutic observations on the group of patients studied

SUMMARY

Paroxysmal hemoglobinuria is a manifestation of late syphilis, it is characterized by the presence of an extremely labile serum auto-hemolysin which is distinct from the Wassermann reacting substance. Union of the hemolysin with the red blood cells occurs only at low temperatures, short chilling is more effective than long periods at low temperatures, the auto hemolysin and the iso-hemolysin are probably identical. CO_2 has been reported to act as a substitute for chilling but such an effect was not demonstrable in the patients of this series. Artificial production of a paroxysm causes hemoglobinemia with or without hemoglobinuria, frequently a sudden drop in the total white count and the percentage of polymorphonuclear cells with a subsequent

leucocytosis and increase of the percentage of polymorphonuclears, an elevation of blood pressure and a rise in the temperature   Intensive anti-luetic treatment may be expected to cause cessation of symptoms, disappearance of the auto-hemolysin and conversion of a positive to a negative Wassermann reaction in the order named

TECHNIQUE

Our routine method of doing the Donath-Landsteiner reaction has been to draw blood from an arm vein, allow it to clot at room temperature, to separate the serum by centrifugalization as soon as coagulation was complete and to set up the test immediately   In a few instances the serum was kept in the ice box over night and the test set up 18 to 24 hours after drawing the blood   The rapid deterioration of the lysin of some of these patients even at ice box temperature, however, makes it advisable to set up the test as soon as the serum has been separated   A 5 per cent suspension of red blood cells was used unless there was a special reason for a heavier or lighter suspension   The suspension was prepared by washing citrated blood three times with a large volume of 0 85 per cent NaCl and suspending the packed cells in 0 85 per cent NaCl   Owing to the thermolability of some of these lysins the serum was used without heating, but to insure the presence of adequate complement fresh pooled guinea pig serum diluted 1 10 was added   The volume was made up to 0 5 cc with 0 85 per cent NaCl   The chilling was accomplished by placing the tubes in finely crushed ice   The usual time for chilling has been 10 minutes   The warming of the tubes was done by placing them in a water bath at 37°C

Six protocols illustrating the technical procedures followed in studying some of the questions referred to in the text are included   These are representative of a large number of similar experiments performed during the period of nine years that the patients of this series have been studied

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A QUANTITATIVE METHOD FOR THE ESTIMATION OF PEPSIN

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The quantitative estimation of ferments is beset by many difficulties which do not obtain in the case of substances which can be directly weighed or measured   Since, in the present state of our knowledge, ferments can only be recognized through their effects, and since these effects vary greatly with many conditions, it has been difficult to develop standard methods which possess reasonable accuracy and at the same time are not too cumbersome

The effects of ferment action vary with the time over which the reaction proceeds, the concentration of the substrate and the concentration of the enzyme as well as with other factors   Most of the methods have been based on the principle of varying one factor while others are kept constant, and the strength of the enzyme is usually expressed in terms of the degree to which the substrate has been effected   The details of the many procedures which have been devised may be read in Oppenheimer's elaborate treatise (1) or in the briefer compendium of Waksman and Davison (2)   With regard to pepsin the usual procedure has been to allow decreasing quantities of ferment (gastric juice) to act on a uniform amount of substrate (such as a soluble protein) over a standard length of time and to determine the smallest amount of ferment which completely digests (as digestion may be defined) the unit of substrate   Thus, if 0 01 cc of ferment completely digests the unit of substrate it is assumed that 1 cc of ferment has the power of digesting 100 units   As various workers have shown, however, this assumption is entirely erroneous (3) since activity does not vary in direct proportion to the concentration of ferment   Methods based on this general plan must therefore be discarded   Northrup and Hussey recently have pointed out (4) that the time required to cause a given percentage change in the viscosity

of a solution of substrate is nearly inversely proportional to the amount of enzyme present   On this basis they have developed a practical method for the measurement of peptic or tryptic activity Special precautions are necessary, however, in the preparation of the substrate

The present method, which was devised during the course of studies of peptic activity of gastric juice subsequently to be reported, is in accord with the established principles of ferment action and in practice has been found to be reasonably simple and accurate   In brief, increasing dilutions of ferment (gastric juice) are allowed to act on a uniform amount of substrate (soluble protein) for a uniform length of time (one-half hour at 37°C)   Instead of determining the greatest dilution which gives complete digestion the percentages of substrate digested by various dilutions of ferment are estimated   From these figures a curve can be constructed which shows at its peak the maximum degree of ferment activity under the conditions of the experiment

PROCEDURE

A standard solution of protein (substrate) is prepared as follows 2 grams of edestin (Special Chemicals Company, Waukegan, Ill) are placed in a small beaker   To this is added, without stirring, 50 cc of 0 3 N hydrochloric acid   The mixture is placed in a water bath (100°C) for one-half hour   Occasionally a minute residue of undissolved edestin remains   This is filtered off with gauze and dissolved in 10 cc of acid which is then added to the edestin already dissolved   A few drops of thymol blue are next introduced as indicator and 20 cc N/10 barium hydroxide are added   The mixture is then placed in a 100 cc volumetric flask which is filled to the 100 cc mark by adding N/100 HCl   The reaction is tested colorimetrically and adjusted so that the final mixture is a 2 per cent solution of edestin with a pH of approximately 2 0   This mixture may be kept on ice apparently unaltered (see below) for at least several weeks   It should be well shaken before being used

In a series of small test tubes increasing dilutions of ferment (gastric juice) in N/100 HCl are set up   The ratio of successive dilutions may be as 1 2 4, etc , or otherwise as desired   In any case the total
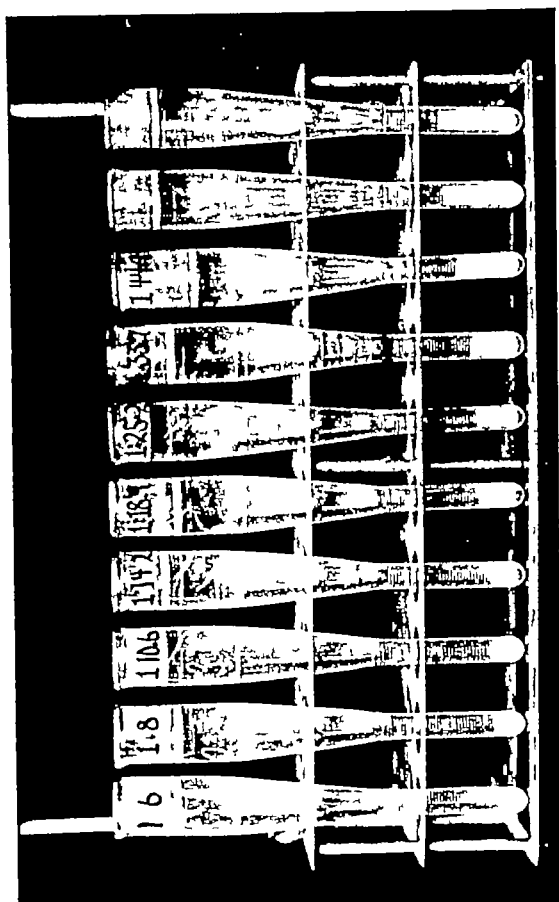
FIG 1 PHOTOGRAPH OF RACK OF TUBES FROM TEST SHOWN IN CHART 5, A

volume of diluted ferment in each tube is 1 0 cc   Five tubes containing 1 0 cc of N/100 HCl but no ferment are added to the series as controls   To each tube 1 cc of the edestin solution is next added and the tubes are then shaken and placed in the water bath at 37°C for exactly one-half hour   Throughout the procedure accurately calibrated volumetric pipettes are used and the digestion is carried out at approximately pH 2 0   After incubation 1 cc of 15 per cent trichloracetic acid is added to each tube   Unless complete digestion has taken place the remaining unsplit protein is precipitated   The contents of each tube are next transferred to a specially graduated centrifuge tube and the volume is made up to a total of 6 5 cc   The tubes have a capillary stem accurately graduated to 0 01 cc [1]   The tubes are next allowed to stand for several hours or preferably over night at room temperature to allow swelling of the precipitate to come to an equilibrium and they are then centrifuged at 1600 revolutions for exactly 10 minutes   An automatic timing device attached to the centrifuge is very convenient   The bulk of the precipitate in the control tubes and in the test tubes is then read on the scale and the degree of digestion which has taken place with each dilution of ferment can be expressed in percentages in relation to the control If, for example, the average of the control tubes which contained no ferment shows a precipitate to the 0 30 mark and if the tube containing 1 cc of ferment shows precipitate to the 0 15 mark and if the control tube represented 300 mgm of edestin, then 1 cc of ferment has digested $\frac{0\,15}{0\,30} = \frac{1}{2} = 150$ mgm of edestin. The calculations can best be demonstrated by an illustrative protocol

Experiment 1   Increasing dilutions of a solution of commercial pepsin (Squibb's) in N/100 HCl were set up as shown in the protocol (table 1)   To each tube 1 cc of a 2 per cent solution of edestin in N/100 HCl was then added, the tubes were incubated in the water bath at 37°C for 30 minutes, trichloracetic acid was added and the tubes were centrifuged   The second column (readings of protein precipitated) shows the reading of the height of the undigested precipitated protein in the capillary stem of the graduated centrifuge tube   The figures in the third and fourth columns are self-evident

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[1] This tube devised by Dr Thomas Addis for measuring small amounts of precipitate is made by the A H Thomas Company. (See fig 1)

TABLE 1

Data from a solution of commercial pepsin (Squibb)

	Dilutions of pepsin in N/100 HCl								
	1 6	1 8	1 106.6	1 14.2	1 18.9	1 25.2	1:33.7	1 44.9	1 59.8
Readings of precipitated protein on graduated scale of centrifuge tube	0 060	0 071	0 080	0 094	0 104	0 131	0 174	0 211	0 243
Amounts of protein digested (difference between control and specimen reading)	0 190	0 179	0 170	0 156	0 146	0 119	0 076	0 039	
Percentage of protein digested $\left(\dfrac{\text{Amount digested}}{\text{Control}}\right)$	76 0	71 6	68 0	62 4	58 4	47 6	30 4	15 6	
Mgm. edestin digested by 1 cc. of 1 per cent pepsin solution under varying conditions (concentration edestin × per cent digested × dilution of specimen)	91 2	114 6	145 0	177 3	221 3	240 3	205 0	140 0	
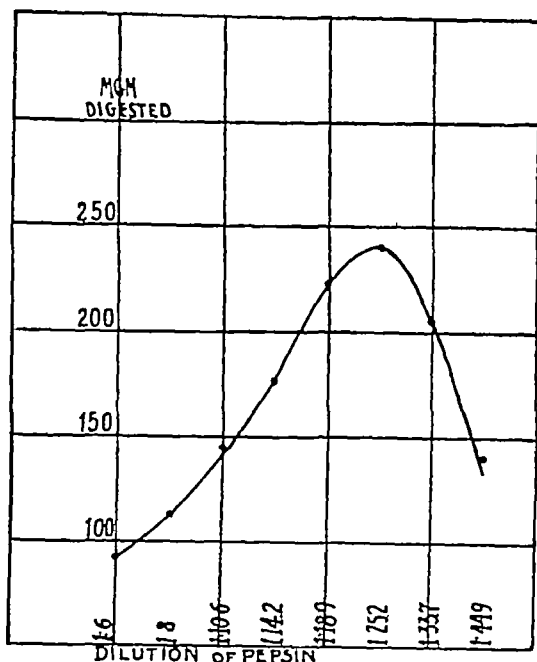
CHART 1 CURVE SHOWING AMOUNTS OF EDESTIN (MILLIGRAMS) DIGESTED BY
1 CC OF 1 PER CENT PEPSIN SOLUTION UNDER VARIOUS
CONDITIONS OF DILUTION
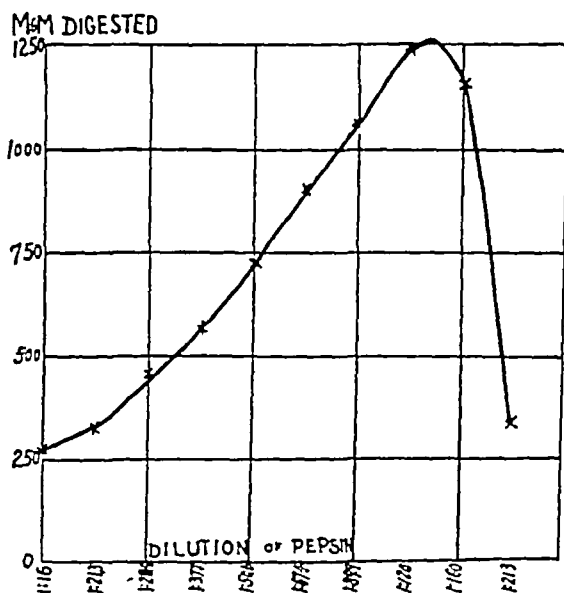
CHART 2 CURVE SHOWING AMOUNTS OF EDESTIN (MILLIGRAMS) DIGESTED BY
1 CC OF GASTRIC JUICE UNDER VARIOUS CONDITIONS OF
DILUTION

50

TABLE 2

Data from human gastric juice obtained after histamine stimulation

	Dilutions of pepsin in N/100 HCl									
	1 16	1 21.3	1 28.4	1 37 7	1 50 6	1 67 4	1 89 9	1 120	1 160	1 213
Readings of precipitated protein on graduated scale of centrifuge tube	0 040	0 042	0 050	0 060	0 070	0 080	0 105	0 120	0 160	0 230
Amounts of protein digested (difference between control and specimen reading)	0 210	0 208	0 200	0 190	0 180	0 170	0 155	0 130	0 090	0 020
Percentage of protein digested $\left(\dfrac{\text{Amount digested}}{\text{Control}}\right)$	84 0	83 2	80 0	76 0	72 0	68 0	62 0	52 0	36 0	8 0
Mgm edestin digested by 1 cc. of 1 per cent pepsin solution under varying conditions (concentration edestin × per cent digested × dilution of specimen)	268 8	354 2	454 9	573 0	728 4	917 0	1114 0	1248 0	1152 0	340 9

The last column gives the actual number of milligrams of edestin digested in each tube multiplied by the dilution of pepsin so that the final results are all expressed in terms of digestive power of 1 cc of undiluted 1 per cent pepsin solution   They are shown graphically in chart 1 by plotting the total amount of edestin digested by 1 cc of
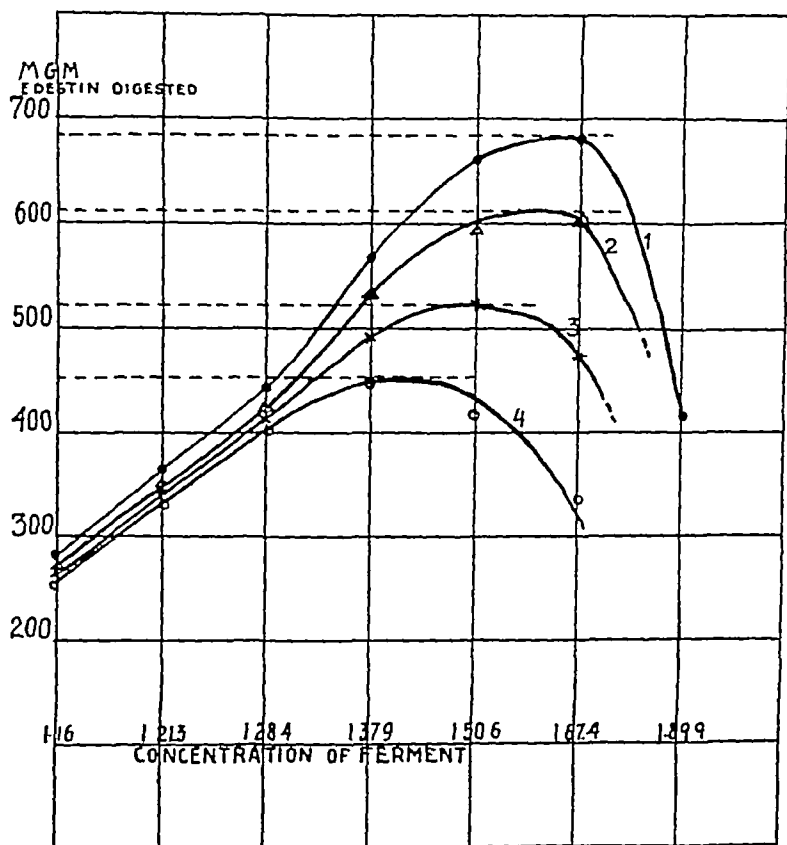
CHART 3   CURVES SHOWING TITRATION OF PEPSIN IN GASTRIC JUICE OBTAINED
BEFORE AND AFTER HISTAMINE STIMULATION

pepsin in various dilutions against the dilution   It is evident that as the dilution of pepsin is increased relatively more digestion takes place until a certain optimum point (the peak of the curve) is reached Beyond this peak further dilution of pepsin is followed by an abrupt lessening of digestive power   If one determines by the above method

enough points to construct a curve it is evident that the high point will indicate the optimum digestive power of the specimen under consideration and will allow comparison with other specimens examined in the same way

Experiment 2   Chart 2 and table 2 show the results of another experiment made with a specimen of gastric juice obtained after histamine stimulation from a normal human stomach   The shape of the curve is similar to that obtained in experiment 1, and indicates that under optimum conditions 1 cc of the gastric juice in question digests about 1250 mgm of edestin   One cubic centimeter of a 1 per cent solution of commercial pepsin, on the other hand (experiment 1), digested only about 242 mgm. of edestin   The activity of the two specimens may therefore be compared, the relation being as 242 1250

In work with normal human gastric juice we have found that in order to construct a satisfactory curve ten tubes are necessary, beginning with a dilution of 1 16 which is increased by 1/4 in each successive tube   In anacidity cases in which pepsin is diminished it is necessary to begin with the undiluted juice, but increasing dilutions in the ratio of 1 2 are adequate   Chart 3 shows the results with specimens of pure gastric juice obtained from the same person before and after histamine stimulation.   Curve 1 is from a specimen before histamine, curves 2, 3 and 4 are from specimens obtained 30, 40 and 50 minutes after histamine.   The optimum digestive power of 1 cc of juice from the various specimens is, in terms of milligrams of edestin, 682, 611, 521 and 452, figures which may be directly compared

CONTROL TESTS

1 *Repeated tests with the same substrate to test its stability*   A lot of substrate was prepared according to the method outlined above.   At weekly intervals it was tested against a solution of pepsin freshly prepared from the commercial powder   Chart 4 shows the results of the three tests which were practically identical   One may conclude that the substrate remains essentially unaltered over a period of at least two weeks

2 Two lots of substrate were prepared and tested with the same pepsin solution at the same time   The results shown in chart 5
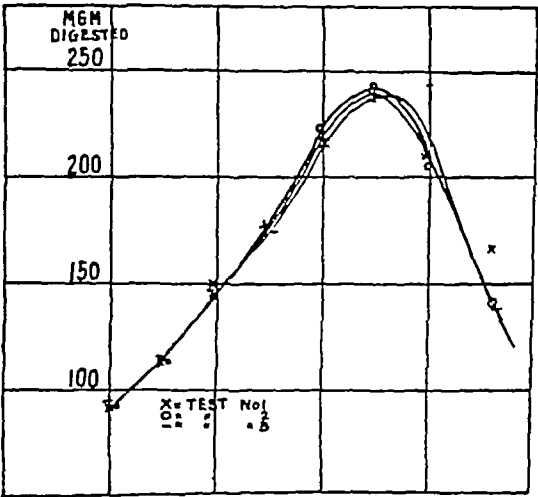
CHART 4   TITRATION OF PEPSIN AGAINST THE SAME LOT OF SUBSTRATE AT WEEKLY
INTERVALS
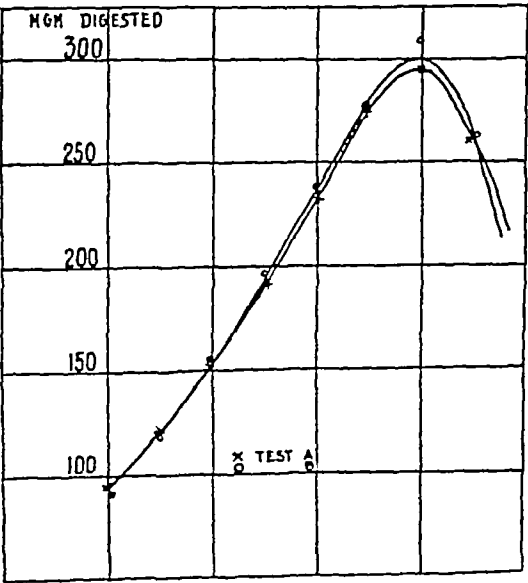
CHART 5   TITRATION OF PEPSIN AGAINST TWO LOTS OF SUBSTRATE

indicate that various lots of substrate are essentially identical and may therefore be used in comparative tests of peptic activity

It is difficult to estimate in mathematical terms the exact degree of accuracy of the method   As is the case with all ferment titrations, unless the most accurate physical chemical methods are employed, a certain amount of error is unavoidable   Our controls indicate that this error is usually not over five per cent

SUMMARY

A method for comparing the peptic activity of specimens of gastric juice which eliminates certain fundamental errors of some of the older methods is described. In the following paper the results of the application of the method in the study of gastric physiology are exposed.

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QUANTITATIVE MEASUREMENTS OF PEPSIN IN GASTRIC JUICE BEFORE AND AFTER HISTAMINE STIMULATION

By W SCOTT POLLAND AND ARTHUR L. BLOOMFIELD

(*From the Department of Medicine, Stanford University Medical School, San Francisco*)

While the chloride and other inorganic constituents of the gastric juice have been thoroughly studied by many workers, few observations on pepsin are on record. The inadequacy of knowledge about this substance is doubtless due to the lack of suitable methods inasmuch as accurate pepsin determinations, as a rule, have not been available in the clinic In another paper (1) we have described a quantitative method for the estimation of pepsin which has been found to be serviceable and fairly accurate and the present report deals with the application of this procedure to the study of gastric secretion in patients

During the course of analyses of pure gastric juice, obtained both with the stomach at rest and after histamine stimulation, it was found (2) that the secretion of various constituents did not run parallel with one another Concentration of chloride and total output of chloride rose after stimulation but concentration of nitrogen and of fixed base fell although the total output was somewhat increased at the height of secretion With regard to pepsin, then, we desired to determine (a) the actual amounts put out by normal people and those with gastric disease, and (b) whether there was a quantitative relation between the curve of pepsin secretion and that of other elements such as nitrogen No data bearing on either of these points were found in the literature since such pepsin estimations as are recorded have usually been made on mixtures of gastric juice and test meal and not on pure gastric juice The true relations are therefore obscured Lim, Matheson and Schlapp (3) report some observations on pepsin before and after histamine stimulation by estimating the time necessary to digest an egg white suspension They state that the concen-

tration is usually little altered after stimulation although it is some-
times greatly increased and that no definite relationship has been
found with the other constituents of the gastric juice This is at
variance with our findings

PROCEDURE

The subjects were all in bed in the hospital After an over-night
fast, a duodenal tube was inserted into the stomach and the fasting
contents were withdrawn The total secretion over ten-minute
periods before and after an injection of histamine (0 1 mgm per 10

TABLE 1

Data from Case 1

Volume of speci-men	Appearance	Titratable acidity		Nitrogen concen tration	Pepsin	
		Free	Total		Mgm edestin digested by 1 cc. of juice	Total mgm di gested per 10 minute period
cc				*mgm per 100 cc*		
35	Clear—some mucus (fasting contents)					
13	Clear, limpid, some mucus	25	35	90 8	735	9,490
Histamine 0 6 mgm						
10	Clear, thin, some mucus	85	98	106	1,540	15,300
39	Clear, thin, some mucus	114	120	59 8	1,240	48,750
32	Clear, thin, some mucus	126	132	42 2	790	25,600
	Part of specimen bile stained	128	132		710	
24	Clear	128	132	42 2	690	16,500

kilos body weight) was then collected exactly as previously described
(2) The following determinations were made (*a*) titratable acidity,
(*b*) pepsin, and (*c*) total nitrogen Titratable acidity was determined
with di-methyl in the usual way, total nitrogen (Kjeldahl) is expressed
in milligrams per 100 cc , and pepsin in terms of the number of milli-
grams of edestin digested by one cubic centimeter of gastric juice in
30 minutes

RESULTS

Case 1 A man, age 27, was convalescent from a mild acute
enteritis He was to all intents and purposes well and had never had

any symptoms of gastric disease   The results of the examination are
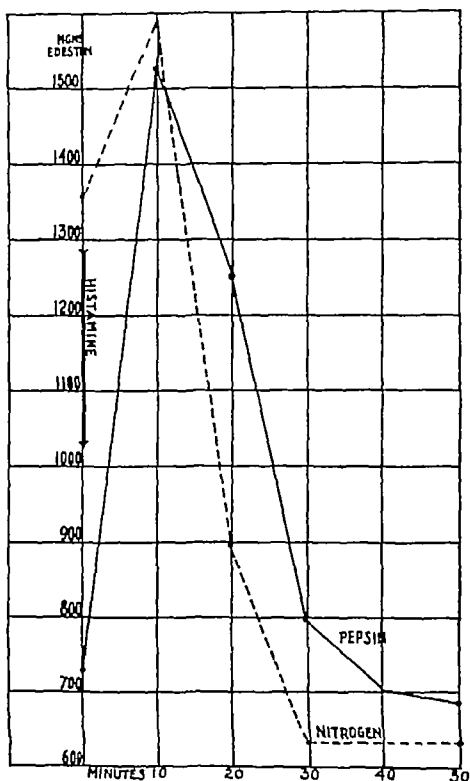shown in table 1 and chart 1   The volume of secretion, the acid

CHART 1   CURVES OF NITROGEN AND PEPSIN CONCENTRATIONS FROM CASE 1 AT
TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

values, and the nitrogen values which show the typical fall in con-
centration after stimulation were all normal   The degree of peptic

activity is measured in terms of milligrams of edestin digested by 1 cc of juice   It is seen that the concentration of pepsin not only falls after stimulation in spite of increased volume of secretion, but that the curve of pepsin concentration at various intervals almost parallels that of nitrogen when both are reduced to a corresponding scale by multiplying the nitrogen values (milligrams per 100 cc) by 15   Unless otherwise indicated, nitrogen values are expressed in this way in all the charts

TABLE 2

Data from case 2

Volume of specimen	Appearance	Titratable acidity		Nitrogen concentration	Pepsin	
		Free	Total		Mgm edestin digested by 1 cc of juice	Total mgm digested per 10 minute period
cc				*mgm per 100 cc*		
20	Colorless, mucoid					
18	Colorless, mucoid	45	69	102 0	1,730	31,140
Histamine 0.5 mgm						
15	Thin, colorless, some mucus	86	107	81 0	1,610	24,150
18	Thin, colorless, some mucus	115	128	58 5	1,030	18,540
19	Thin, colorless, some mucus	120	131	49 5	860	16,340
15	Thin, colorless, some mucus	126	138	61 5	977	14,620
11	Thin, colorless, some mucus	118	130	63 0	1,010	11,110

Case 2   A man, age 24, East Indian, entered the hospital for nervousness   No evidences of organic disease of any sort were found   The results of the examination are shown in table 2 and chart 2   The volume of secretion, the acidity and the nitrogen output were all normal   As in case 1, the concentration of pepsin fell markedly after stimulation and paralleled the fall in nitrogen concentration very closely

Case 3   A man of 23 years, entered the Hospital for minor complaints and showed no evidence of organic disease   The volume of gastric secretion was rather high but not abnormal, acidity and nitrogen were within the usual range   Here again, after stimulation,
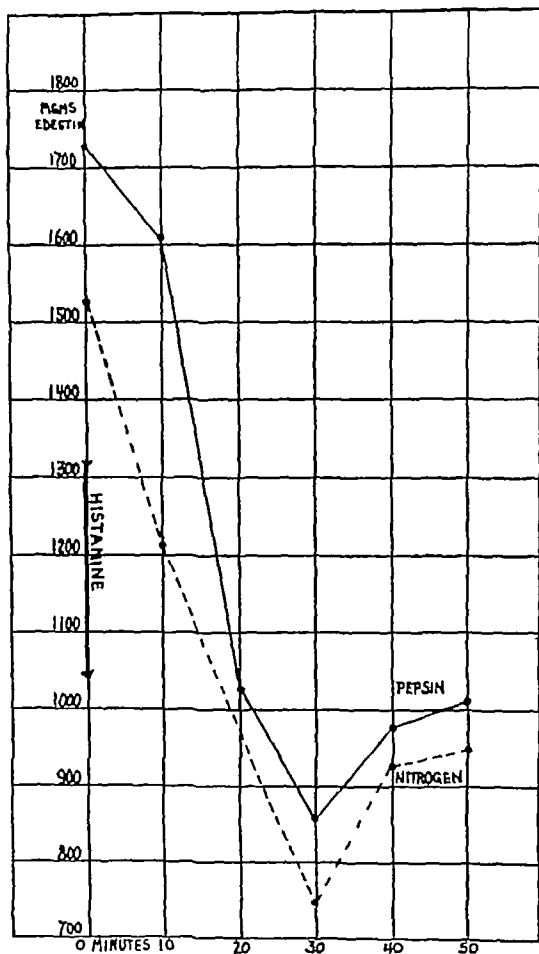
CHART 2 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 2 AT
TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

there was a striking fall in concentration of pepsin which ran parallel to the fall in nitrogen (table 3, chart 3)

Case 4   A man, age 19, had disseminated lupus erythematosus There were no gastric symptoms nor evidences of disease of the stomach   The volume of secretion, acidity and concentration of nitrogen were within normal limits   In this case again the concentration of pepsin fell after stimulation and paralleled the nitrogen concentration although the curves do not correspond as closely as in

TABLE 3

Data from case 3

Volume of speci men	Appearance	Titratable acidity		Nitrogen concen tration	Pepsin	
		Free	Total		Mgm edestin digested by 1 cc. of juice	Total mgm di gested per 10 minute period
cc				*mgm per 100 cc*		
23	Slightly bile tinged fluid some mucus					
8	Colorless—some mucus	35	52	112 5	1,495	11,960
Histamine 0 6 mgm						
12	Colorless—considerable mucus	75	90	127 5	2,075	24,900
23	Water clear fluid	110	116	58 5	1,220	28,060
42	Water clear fluid	121	126	46 5	720	30,240
32	Water clear fluid	118	122	36 0	605	19,200
31	Water clear fluid	103	112	46 5	660	20,460

the three previous cases   The maximum concentration of pepsin, furthermore, is considerably lower than in cases 1, 2 and 3

Case 5   A man of 32 had a penetrating ulcer of the lesser curvature of the stomach   The acidity was within normal limits but the volume of gastric secretion was distinctly high   The pepsin concentration again runs parallel to the nitrogen concentration but is lower than in the normal cases 1, 2 and 3   The low concentration of pepsin is largely explained by the high volume of secretion, the total output of pepsin is not low (see chart 9)

Case 6   A man, age 32, was convalescent from a mild attack of influenza   There were no digestive symptoms and the temperature

had been normal for several days at the time of the test   The findings on gastric analysis were of interest since the acid values were distinctly low, while the volume of secretion was well above the average   Fur-
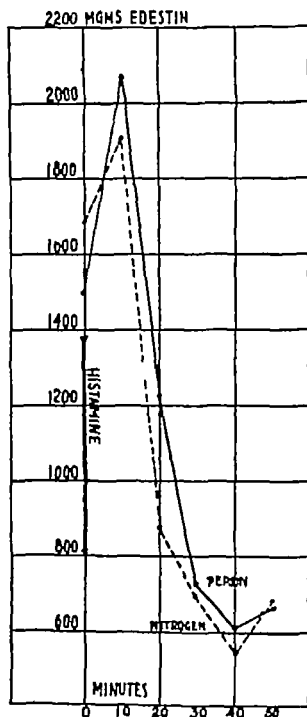

CHART 3 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 3 AT TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

thermore, the gastric juice was unusually viscid and it was suspected that the nitrogen values would be high   The concentration of pepsin was distinctly lower than in cases 1, 2 and 3, but showed the usual drop

TABLE 4

Data from case 4

Volume of speci men	Appearance	Titratable acidity		Nitrogen concen- tration	Pepsin	
		Free	Total		Mgm edestin digested by 1 cc. of juice	Total mgm di gested per 10 minute period
cc				mgm per 100 cc		
50	Bile stained, mucoid					
22	Thin fluid, slightly bile tinged, mucoid	35	50	75 0	680	14,960

Histamine 0 6 mgm						
16	Thin fluid, faint brownish (blood) tinge	35	50	84 0	950	15,200
20	Thin fluid, faint brownish (blood) tinge	110	110	53 4	870	17,400
26	Thin fluid, faint brownish (blood) tinge	135	145	42 6	750	19,500
16	Thin fluid, faint brownish (blood) tinge	130	140	43 5	530	8,480
10	Thin fluid, faint brownish (blood) tinge	118	125	55 0	810	8,100

TABLE 5

Data from case 5

Volume of speci men	Appearance	Titratable acidity		Nitrogen concen tration	Pepsin	
		Free	Total		Mgm edestin digested by 1 cc. of juice	Total mgm di gested per 10 minute period
cc				mgm per 100 cc		
30	Thin fluid, very slight bile tinge—some mucus					
22	Colorless fluid, some mucus	94	100	52 5	365	8,030

Histamine 0 6 mgm						
26	Water clear fluid	86	93	29 4	405	10,530
38	Water clear fluid	119	124	43 5	595	22,610
55	Water clear fluid	126	130	35 1	350	19,250
45	Water clear fluid	128	133	28 5	345	15,525
35	Water clear fluid	118	125	27 9	290	10,150

after stimulation   The curve also ran parallel with nitrogen concentration but when the nitrogen values were charted as in the previous

CHART 4 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 4 AT TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

cases (N mgm per 100 cc $\times$ 15) a wide gap between the two curves is seen   In other words, while a relation between nitrogen and pepsin seems likely there is relatively much more nitrogen present than usual

DISCUSSION OF CASES 1-6

The first point which becomes evident from the preceding charts is the necessity of interpreting the concentration of pepsin in relation to the secretory activity of the stomach   Clearly, a determination of pepsin on a single specimen aspirated after a test meal gives only the most rudimentary sort of information   In case 3, for example, the digestive power of a cubic centimeter of juice was nearly 2100 mgm

CHART 5 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 5 AT TEN-MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

of edestin 10 minutes after stimulation, 30 minutes later it was only 600 mgm   The most striking point in all the curves is the fall in concentration of pepsin at the height of secretion   This is to some extent explained by dilution from increased output of water at this time but as appears from chart 9 the total amount of pepsin secreted is actually increased   The actual values both for concentration and total output of pepsin vary considerably in the six cases   Many

more examinations would be necessary to define accurately normal standards but the limits are indicated.

The parallelism between the curves of pepsin and nitrogen concentration suggests strongly that pepsin is secreted in combination or

TABLE 6

Data from case 6

Volume of specimen	Appearance	Titratable acidity		Nitrogen concentration	Pepsin	
		Free	Total		Mgm. edestin digested by 1 cc. of juice	Total mgm. digested per 10 minute period
cc				mgm per 100 cc		
70	Colorless fluid—very mucoid					
21	Colorless fluid—very mucoid	8	24	109	685	14,385
Histamine 0.5 mgm						
28	Colorless fluid—very mucoid	22	35	100	690	19,320
40	Colorless fluid—very mucoid	56	68	116	860	34,400
47	Colorless fluid—very mucoid	63	75	81 4	610	28,670
50	Colorless fluid—very mucoid	52	63	86 0	520	26,000
36	Colorless fluid—very mucoid	64	75	69 5	450	16,200

TABLE 7

Relation of acidity to pepsin concentration of gastric juice

Case	Highest acidity	Highest concentration pepsin
4	135	870
1	128	1,540
5	128	595
2	126	1,730
3	121	2,075
6	63	860

in association with some nitrogenous body   It is equally clear that there is no close correlation between the acidity of the gastric juice and its peptic activity (see table 7)
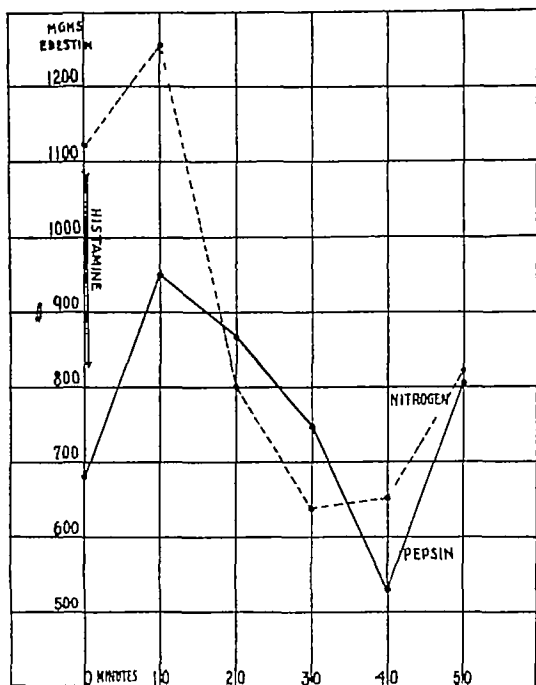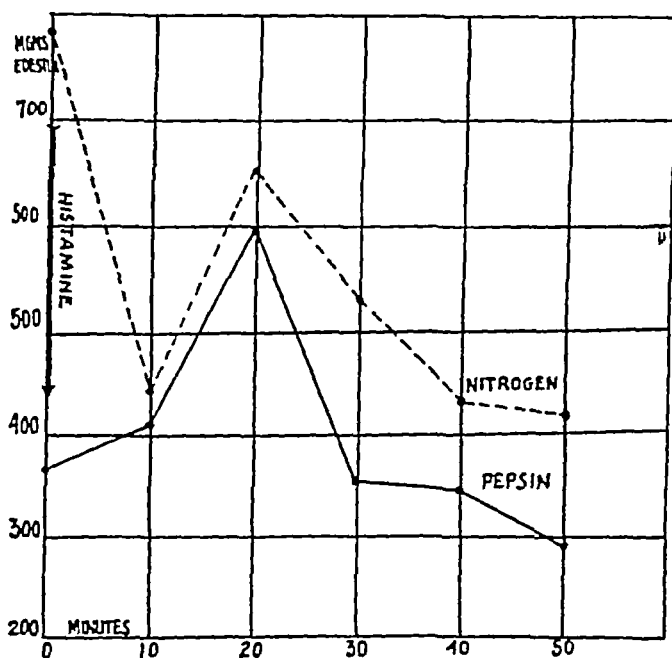
CHART 6   CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 6 AT
TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

TABLE 8

Data from case 7

Volume of specimen	Appearance	Titratable acidity		Nitrogen concentration	Pepsin	
		Free	Total		Mgm. edestin digested by 1 cc. of juice	Total mgm. digested per 10 minute period
cc				mgm per 100 cc.		
40	Turbid colorless fluid					
29	Colorless thin mucoid fluid	12	25	45 3	380	11 020

Histamine 0 6 mgm.						
13	Water clear fluid	27	37	32 1	318	4,111
17	Water clear fluid	43	53	43 5	270	4,624
16	Water clear fluid	60	70	50 1	320	4,960
15	Water clear fluid	27	40	54 9	170	2,580
7	Water clear fluid	23	41	47 3	265	1,955

TABLE 9

Data from case 8

Volume of specimen	Appearance	Titratable acidity		Nitrogen concentration	Pepsin	
		Free	Total		Mgm edestin digested by 1 cc. of juice	Total mgm digested per 10 minute period
cc.				mgm per 100 cc.		
30	Clear colorless mucoid fluid					
7	Clear colorless mucoid fluid	0		104 6	17	112

Histamine 0 6 mgm						
4	Clear colorless mucoid fluid	0		105 6	60	240
3 5	Clear colorless mucoid fluid	0		126 6	50	175
3	Clear colorless mucoid fluid	0		142 0	85	240
3	Clear colorless mucoid fluid	0		137 3	50	150

OBSERVATIONS IN GASTRIC DISORDERS

The following observations, made in patients with evidence of deficient gastric secretion, bring out certain points of importance in contrast to the preceding group
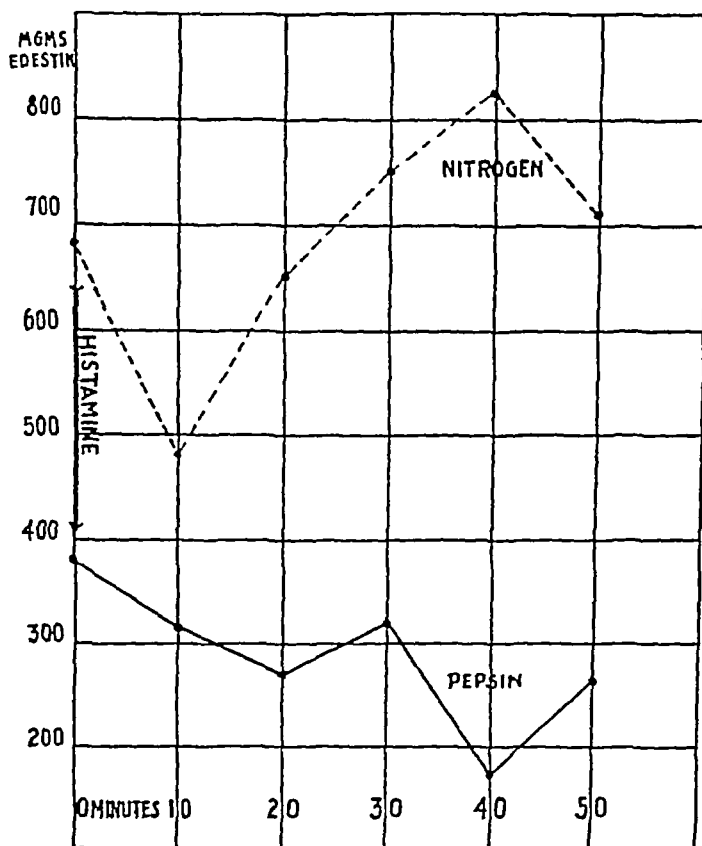
CHART 7   CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 7 AT
TEN-MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

Case 7   A man, age 57, was under treatment in the Hospital for sprue   Routine fractional gastric analyses showed the presence of HCl in the stomach contents but only in small amount   The observations are summarized in table 8 and chart 7   The gastric acidity even after histamine stimulation is low but the volume of secretion is
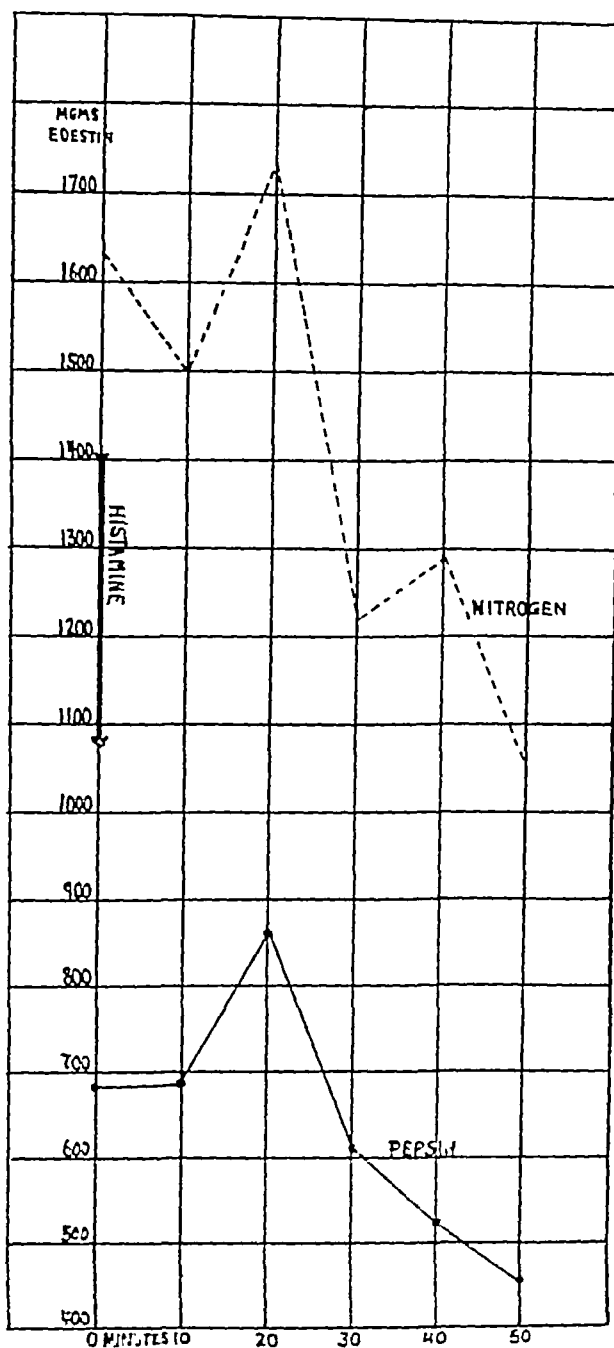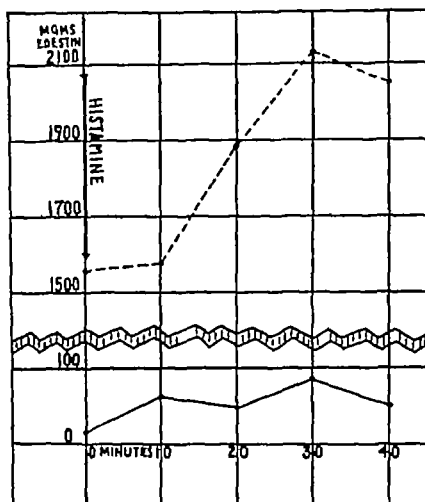
CHART 8 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 8 AT
TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

TABLE 10

Data from case 9

Volume of speci-men	Appearance	Titratable acidity		Nitrogen concen-tration	Pepsin	
		Free	Total		Mgm. edestin digested by 1 cc. of juice	Total mgm. di-gested per 10 minute period
cc.				*mgm. per 100 cc*		
30	Fasting contents foul mucus, pus and blood	0				
5	Pale brown foul thin fluid	0			58+	290+
Histamine 0.5 mgm.						
5 5	Pale brown foul thin fluid	0			57	313
0 5	Pale brown foul thin fluid	0			20	80
3 5	Pale grey brown mucoid, foul	0				
0						

not remarkable   The concentration of pepsin is much lower than in the normal cases and it neither shows the typical drop after stimulation
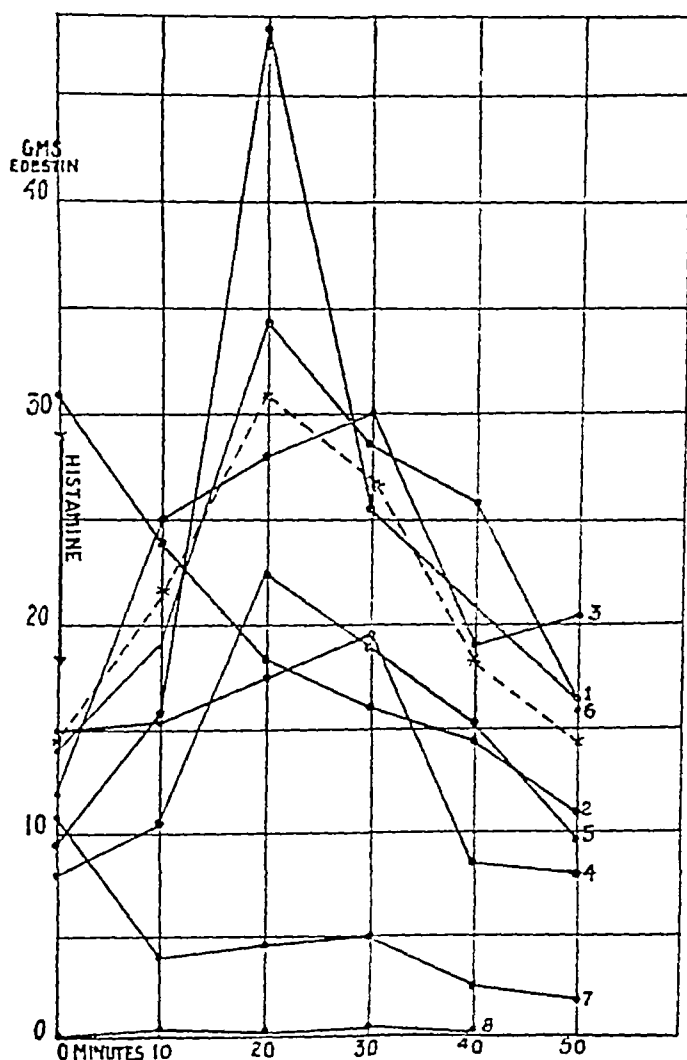
CHART 9 TOTAL OUTPUT OF PEPSIN PER TEN-MINUTE PERIOD BEFORE AND AFTER HISTAMINE

nor does it follow the nitrogen curve   The total output of pepsin is also low

Case 8   A woman, age 57, had been under observation for four years with pernicious anemia   The findings were typical and recently there had been a good response to liver feeding although at the time of the present test there was an anacidity even after histamine stimulation   The results are shown in table 9 and chart 8   The usual almost complete absence of secretion is evident   However, in spite of the anacidity the small amount of juice obtained showed definite though diminished peptic activity, the concentration of pepsin being roughly one tenth to one twenty-fifth normal   There was no relation between the nitrogen and pepsin curves and the total output of pepsin (chart 9) was, of course, small—approximately one hundredth normal

Case 9   A man, age 72, had an advanced cancer of the stomach with ascites and masses in the liver   When examined it was possible to obtain only small amounts (see table 10) of a brownish foul fluid containing considerable granular debris   The specimens were centrifuged and the supernatant fluid tested   The fluid itself contained a considerable amount of protein precipitable by trichloracetic acid This was measured and a correction was made   The table shows that even in the presence of an advanced sloughing carcinoma with total absence of acid, pepsin was still present in readily demonstrable amounts

SUMMARY

A series of observations on the application in the clinic of a quantitative method of pepsin estimation are reported   The curves of pepsin concentration and of total pepsin output before and after stimulation by histamine are described   In normal people the concentration of pepsin falls markedly after stimulation and follows closely the curve of nitrogen concentration   The total output of pepsin is usually increased

Aberrations from the normal are illustrated and it is pointed out that even in pernicious anemia of long standing and in advanced cancer of the stomach, some pepsin may still be secreted   Estimation of pepsin may turn out to be a more delicate index of gastric damage, in certain types of cases, than determinations of acidity

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STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM[1]

II The Calcium Excretion of Normal Individuals on a Low Calcium Diet, also Data on a Case of Pregnancy[2]

By WALTER BAUER,[3] FULLER ALBRIGHT,[4] AND JOSEPH C AUB

(From the Medical Clinic of the Massachusetts General Hospital, Boston)

As a result of the lead studies carried on in this laboratory (1), (2), it has become increasingly apparent that it is important to be able to alter the deposition or mobilization of calcium phosphate in bone. A greater knowledge of the factors which influence calcium balance has therefore been sought The decision as to the method of approaching this problem involved the following considerations In the first place it was obvious that very little could be learned by studying the serum calcium alone, as this merely shows the height of the "calcium stream" but gives no indication as to the direction of its flow, into the excretory channels or into the bones So it at once was clear that analyses of calcium intake and output would be a necessary addition to the blood studies Furthermore, inasmuch as calcium is excreted into the bowel as well as into the urine, any figure obtained for fecal calcium would consist of two components, the calcium which had passed through the intestines unabsorbed and the calcium which had been absorbed and re-excreted The latter of these alone can be thought of as actively taking part in the calcium metabolism A way of overcoming this difficulty would be to have a diet with a calcium intake of zero but adequate in every other respect Since this is not practicable we adopted a diet with a calcium intake as low

as possible and still adequate in caloric, protein, and vitamine requirements   By studying the calcium balance of an individual while on such a low calcium intake (about 100 mgm a day), one can deduce fairly well the rate of endogenous calcium exchange   A further advantage of such a technique is that, by having a very low calcium intake, one largely escapes the many variables which influence calcium *absorption*, such as the acid-base values of the diet (3), the amount of fat (4) (5) or of vitamine D in the diet, and the amount of exposure to ultra violet light   Therefore, a low calcium diet has been used in observing the calcium excretion of normal and pathological individuals, in order to determine factors which caused deviations from the normal   It is the purpose of this paper to present the data for the calcium excretion of some normal people

NORMAL VARIABLES WHICH INFLUENCE CALCIUM METABOLISM

Other factors which might influence the calcium excretion are (a) sex, (b) age, (c) weight or body surface, (d) activity of subject, (e) acid-base values of diet, (f) amounts of other cations in intake, and (g) the phosphorus metabolism

Sex plays a small but definite part in the quantitative comparison of the energy metabolism   Unless its effects upon calcium metabolism were of a relatively different magnitude it would be insignificant, because the methods available in determining the calcium metabolism are far less precise   In our studies it so happens that our control determinations are all made on men, whereas many of the pathological conditions studied were in women, so that this is a possible source of a small error in our comparisons

Again, as in energy metabolism, it is not unlikely that age affects in some measure the rate of the calcium metabolism   Of course during the period of bone growth, there must be a marked quantitative effect on the calcium metabolism   In old age the atrophy of bone, as seen by x-ray, suggests a possible alteration in the rate of the calcium metabolism   Aside from these extremes of life, the basal metabolism remains quite constant and it is unlikely that there would be any marked variation in the rate of normal calcium exchange   Our control subjects varied between 19 years and 60 years with an average age of 41 3 years

The size of the individual must play some part in the rate of the calcium metabolism Sherman (6), in an effort to determine the calcium requirement for maintenance in man, found it useful to reduce his figures for the calcium excretion, under various inadequate intakes, to the calcium excretion per seventy kilograms Possibly the calcium excretion per unit of surface area would be theoretically more correct, but we have followed the lead of Sherman and have reduced our calcium excretions and negative calcium balances in our controls to the amount per kilogram of body weight However, for comparative purposes, we use the figures for the average calcium excretion per person per three day period without reference to weight, and then give the average weight of the control subjects

As regards the activity of the subject and its effect on calcium metabolism, there is little known In bone the phenomenon of atrophy of disuse is a very striking and real one, but as far as we know there have been no experiments to show whether this represents an altered calcium metabolism throughout, or merely in the immobilized parts of the body Experiments are being planned to determine this fact All but two of our controls were patients up and about the hospital ward, these two were doctors doing strenuous hospital work

The ingestion of mineral acids has been proven to increase the calcium excretion (1) (7) (8) (9) (10) (11) Diets in which the mineral acid elements predominate over the fixed base elements also lead to decalcification (11) (12) (13) (14) Thus Bogert and Kirkpatrick (12) were able to increase the calcium excretion, especially that in the urine, by changing the food allowance of potato (a basic substance) to rice (an acid substance) The literature leads one to believe that an excess of alkali also leads to decalcification (15) (16) The modus operandi here, however, is possibly due chiefly to decreased absorption because of the increased alkalinity of the intestinal contents (3) For comparative studies like our own, therefore, the acid base balance of the diet should be constant even if not in acid-base equilibrium We have not attempted to balance each diet in this respect, but have been content to use a very limited number of food substances, believing that, in this way, the acid base values would be fairly constant In order to estimate the variation of our diets in this respect we have shown in table 1 and figure 1 the acid and base ele

as possible and still adequate in caloric, protein, and vitamine requirements   By studying the calcium balance of an individual while on such a low calcium intake (about 100 mgm a day), one can deduce fairly well the rate of endogenous calcium exchange   A further advantage of such a technique is that, by having a very low calcium intake, one largely escapes the many variables which influence calcium *absorption*, such as the acid-base values of the diet (3), the amount of fat (4) (5) or of vitamine D in the diet, and the amount of exposure to ultra violet light   Therefore, a low calcium diet has been used in observing the calcium excretion of normal and pathological individuals, in order to determine factors which caused deviations from the normal   It is the purpose of this paper to present the data for the calcium excretion of some normal people

NORMAL VARIABLES WHICH INFLUENCE CALCIUM METABOLISM

Other factors which might influence the calcium excretion are (a) sex, (b) age, (c) weight or body surface, (d) activity of subject, (e) acid-base values of diet, (f) amounts of other cations in intake, and (g) the phosphorus metabolism

Sex plays a small but definite part in the quantitative comparison of the energy metabolism   Unless its effects upon calcium metabolism were of a relatively different magnitude it would be insignificant, because the methods available in determining the calcium metabolism are far less precise   In our studies it so happens that our control determinations are all made on men, whereas many of the pathological conditions studied were in women, so that this is a possible source of a *small error in our comparisons*

Again, as in energy metabolism, it is not unlikely that age affects in some measure the rate of the calcium metabolism   Of course during the period of bone growth, there must be a marked quantitative effect on the calcium metabolism   In old age the atrophy of bone, as seen by x-ray, suggests a possible alteration in the rate of the calcium metabolism   Aside from these extremes of life, the basal metabolism remains quite constant and it is unlikely that there would be any marked variation in the rate of normal calcium exchange   Our control subjects varied between 19 years and 60 years with an average age of 41 3 years

(17) and Clark (18)   The values in diets C, D, and E, except for sulphur and chlorine, were also checked by actual determination, so that the calculated and actually determined values can be compared   The value of each acid or base element was reduced to cubic centimeters of tenth normal solution, phosphoric acid being considered divalent (17)   The total acid value thus obtained was then compared with the total base value and the acid or base balance value noted (see table 1 and figure 1)   It will be seen that all five diets

TABLE 1

Acid-base properties of five typical diets†

	Diet A	Diet B	Diet C		Diet D		Diet E	
			Calculated	Found	Calculated	Found	Calculated	Found
Calcium	56	41	66	52	55	43	35	31
Magnesium	230	168	215		196		118	
Potassium	774	533	882		781		446	
Sodium	495	419	525		400		312	
Total base	1 555	1 161	1,688	2 026	1,432	1,647	911	1,223
Phosphorus*	762	453	735	600	530	440	375	369
Chlorine	454	391	453		380		272	
Sulphur	679	424	793		557		387	
Total fixed acid	1,895	1 268	1,981		1,467		1,034	
Excess of acid	340	107	293		35		123	
Calories	2,874	2,660	3,051		2,345		1,421	

* Calculated as divalent.

† All figures in cubic centimeters of N/10, except calories.

had an excess of acid over base—the most acid diet having a net acid value of 340 cc of tenth normal and the least acid a net acid value of 35 cc of tenth normal   These variations do not seem excessive   The calculated values agree fairly well with the actually determined values   The total base values actually determined are higher than the calculated ones, due probably to the amount of salt allowed in the diet [5]   The total acid-base metabolism is also

[5] The effect of acid and base excesses in the diet have been thoroughly studied and will be reported in a subsequent paper in this series

ments of five diets   Diets A and B are two of the actual diets used by two of the control patients, the patient eating diet A had the highest urinary calcium excretion of any of the control patients, and the patient eating diet B had one of the lowest urinary calcium ex-
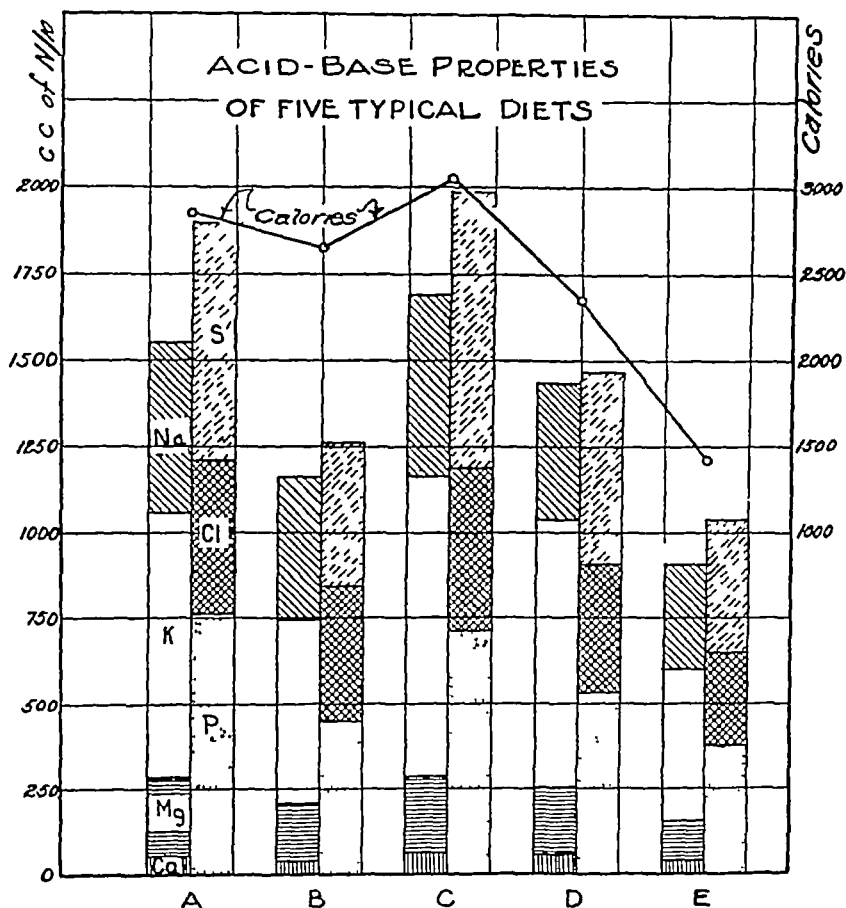
FIG 1

cretions   Thus it was believed that here, if anywhere, we would find a discrepancy between the acid-base values of the diets

Diets C, D, and E happen to have been used in later experiments, though they are similar to those used in these controls   The values for the acid and base elements are taken from Sherman and Gettler

doing hospital work   We have in all forty six three-day periods on these controls   No period was used unless the subject had had a fore period on a low calcium diet (about 100 mgm a day) of at least 36 hours, or unless at least three days had passed since any medication such as potassium iodide had been used   The details of the prepara-
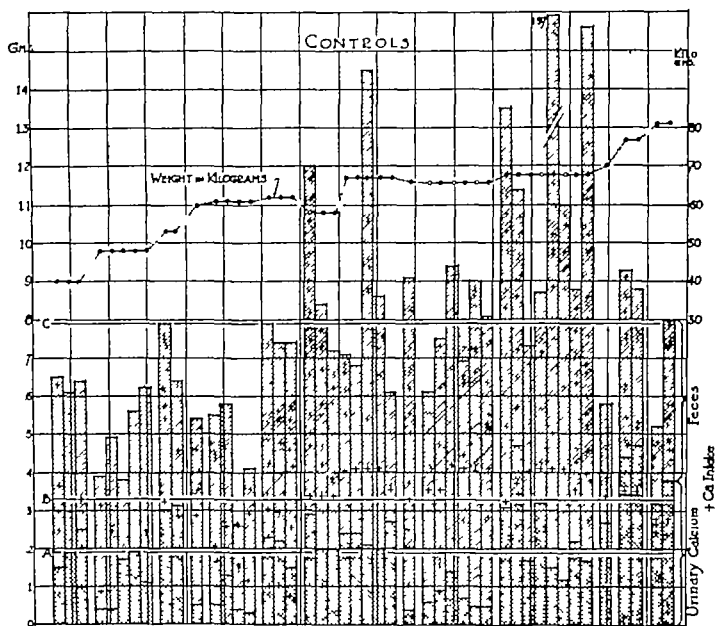
FIG 2 CALCIUM BALANCE OF 13 CONTROL SUBJECTS ON A LOW CALCIUM DIET

Line I represents average urinary calcium excretion, line B average calcium intake, line C average total calcium excretion The distance BC, therefore represents the average negative calcium balance

tion of the diet, the collection of the excreta, and the methods of analysis have been given in a previous paper (24)   The data are presented in table 2 and figure 2   The periods from each subject have been grouped together but they are not necessarily consecutive periods The subjects themselves have been arranged in order of their weights

dependent upon the nitrogen balance of the subject, for if the subject is in negative nitrogen balance, one should add to the acid-base values of the ingested food, the acid-base values of the tissue destroyed   Thus in every subject whose calcium metabolism is being determined, one should know the nitrogen balance   This we have done throughout our studies, except in our controls where only two were studied from this aspect   It may be said, however, that the controls were receiving more than a gram of protein per kilo per day, were not losing weight and therefore a negative nitrogen balance, if present, must have been small

Because of the various inter-relations which the cations, calcium, magnesium, sodium and potassium have (14) (19) (20) (21) (22), in a study of any one of them, it would be desirable that the intakes of all the others be absolutely fixed   To what extent this has been the case in five of our diets can be seen by again referring to table 1 and figure 1

Calcium metabolism is inseparably linked with phosphorus metabolism   Most of the calcium retained in the body is usually assumed to be in the form of tertiary calcium phosphate deposited in the bones Phosphoric acid (ingested either as such or as the acid phosphate) produces marked decalcification (11)   Neutral sodium phosphate injected into the blood stream also causes an increased calcium excretion (23) So, under ideal conditions, quantitative calcium metabolism studies, if to be used for comparison with other experiments, should have a constant phosphorus intake   We have not attempted to do this but have been content with the variations which our small menu allowed Table 1 and figure 1 show the actual variation found in five of our diets   As in the discussion of the acid-base values of the diet, it must be pointed out here that unless the subject is in nitrogen equilibrium, the phosphorus in the diet really consists of the phosphorus ingested plus the phosphorus liberated from the destruction of body protein

EXPERIMENTS

Studies have been made on thirteen individuals   Eleven of these were convalescing from industrial lead poisoning, but were otherwise normal   We have no reason to suppose that lead poisoning affects the rate of calcium metabolism   The other two subjects were doctors

TABLE 2—*Continued*

Subject	Age	Weight	Period	Calcium in grams per 3-day period					Total calcium excretion per kilogram	Negative calcium balance per kilogram
				Urine	Feces	Total excretion	Intake	Balance		
		kgm.							*grams*	*grams*
D M	50	66	6	0 06	0 55	0 61	0 355	−0 25	0 012	0 0060
			7	0 09	0 66	0 75	0 355	−0 39		
			14	0 14	0 79	0 93	0 420	−0 51		
			15	0 07	0 62	0 69	0 411	−0 28		
			16	0 05	0 85	0 90	0 405	−0 49		
			17	0 05	0 76	0 81	0 348	−0 46		
J T	33	68	9	0 31	1 04	1 35	0 326	−1 01	0 018	0 0119
			18	0 47	0 67	1 14	0 372	−0 77		
			41	0 17	0 56	0 73	0 405	−0 33		
			42	0 32	0 55	0 87	0 411	−0 46		
			43	0 15	1 80	1 95	0 411	−1 54		
			44	0 12	0 98	1 10	0 411	−0 69		
			53	0 22	0 66	0 88	0 357	−0 52		
			59	0 17	1 39	1 56	0 399	−1 16		
J M	35	70	2	0 27	0 31	0 58	0 339	−0 24	0 008	0 0034
C H.	25	77	1	0 44	0 48	0 92	0 35	−0 57	0 012	0 0071
			2	0 47	0 41	0 88	0 35	−0 53		
C K.	30	81 4	1	0 32	0 20	0 52	0 28	−0 24	0 008	0 0052
			2	0 38	0 46	0 84	0 23	−0 61		
Average	42 3	67 2		0 19	0 60	0 79	0 33	−0 46	0 012	0 0067

RESULTS

It will be seen from table 2 and figure 2 that there is considerable variation in the calcium excretion not only among different individuals but also among different periods of the same individual This variation could probably be reduced by a more rigid standardization of the aforementioned normal factors which influence calcium metabolism However, these figures give us an indication of the amount of calcium which a normal person will excrete on a low calcium diet and their average will be used for future comparisons Atten-

TABLE 2

Calcium studies in "normal" controls

| Subject | Age | Weight | Period | Calcium in grams per 3-day period | | | | | Total calcium excretion per kilogram | Negative calcium balance per kilogram |
				Urine	Feces	Total excretion	Intake	Balance		
		kgm							*grams*	*grams*
J P	60	40	20	0 15	0 50	0 65	0 299	−0 35	0 016	0 0081
			5	0 19	0 42	0 61	0 290	−0 32		
			9	0 25	0 39	0 64	0 336	−0 30		
W C	19	48	2	0 04	0 35	0 39	0 085	−0 30	0 010	0 0056
			3	0 04	0 44	0 49	0 246	−0 24		
			4	0 17	0 21	0 38	0 246	−0 13		
			5	0 19	0 37	0 56	0 246	−0 31		
			6	0 11	0 51	0 62	0 246	−0 37		
M R	53	53	5	0 30	0 49	0 79	0 322	−0 47	0 013	0 0076
			6	0 31	0 33	0 64	0 300	−0 34		
N D	55	60	13	0 05	0 49	0 54	0 288	−0 25	0 009	0 004
K K	53	61	6	0 05	0 50	0 55	0 208	−0 34	0 007	0 0032
			7	0 13	0 45	0 58	0 258	−0 32		
			26	0 04	0 22	0 26	0 262	0 0		
			27	0 03	0 38	0 41	0 294	−0 12		
M C	50	62	22	0 23	0 56	0 79	0 303	−0 49	0 012	0 0076
			23	0 22	0 52	0 74	0 298	−0 44		
			11	0 15	0 59	0 74	0 252	−0 49		
P M	48	58	31	0 29	0 81	1 10	0 340	−0 76	0 014	0 0078
			39	0 20	0 65	0 85	0 340	−0 51		
			40	0 20	0 52	0 72	0 340	−0 38		
		67	63	0 24	0 47	0 71	0 405	−0 31		
			64	0 24	0 44	0 68	0 411	−0 27		
			65	0 21	1 24	1 45	0 411	−1 04		
			66	0 20	0 66	0 86	0 411	−0 45		
			75	0 27	0 44	0 71	0 357	−0 25		
P S	39	64	34	0 04	0 86	0 91	0 330	−0 58	0 014	0 0091

TABLE 2—*Continued*

Subject	Age	Weight	Period	Calcium in grams per 3-day period					Total calcium excretion per kilogram	Negative calcium balance per kilogram
				Urine	Feces	Total excretion	Intake	Balance		
		kgm							*grams*	*grams*
D M	50	66	6	0 06	0 55	0 61	0 355	−0 25	0 012	0 0060
			7	0 09	0 66	0 75	0 355	−0 39		
			14	0 14	0 79	0 93	0 420	−0 51		
			15	0 07	0 62	0 69	0 411	−0 28		
			16	0 05	0 85	0 90	0 405	−0 49		
			17	0 05	0 76	0 81	0 348	−0 46		
J T	33	68	9	0 31	1 04	1 35	0 326	−1 01	0 018	0 0119
			18	0 47	0 67	1 14	0 372	−0 77		
			41	0 17	0 56	0 73	0 405	−0 33		
			42	0 32	0 55	0 87	0 411	−0 46		
			43	0 15	1 80	1 95	0 411	−1 54		
			44	0 12	0 98	1 10	0 411	−0 69		
			53	0 22	0 66	0 88	0 357	−0 52		
			59	0 17	1 39	1 56	0 399	−1 16		
J M	35	70	2	0 27	0 31	0 58	0 339	−0 24	0 008	0 0034
C H.	25	77	1	0 44	0 48	0 92	0 35	−0 57	0 012	0 0071
			2	0 47	0 41	0 88	0 35	−0 53		
C K.	30	81 4	1	0 32	0 20	0 52	0 28	−0 24	0 008	0 0052
			2	0 38	0 46	0 84	0 23	−0 61		
Average	42 3	67 2		0 19	0 60	0 79	0 33	−0 46	0 012	0 0067

RESULTS

It will be seen from table 2 and figure 2 that there is considerable variation in the calcium excretion not only among different individuals but also among different periods of the same individual   This variation could probably be reduced by a more rigid standardization of the aforementioned normal factors which influence calcium metabolism   However, these figures give us an indication of the amount of calcium which a normal person will excrete on a low calcium diet and their average will be used for future comparisons   Atten-

TABLE 3

Calcium studies on low calcium diets (taken from the literature)

Author	Weight	Calcium in grams per 3-day period					Total calcium excretion per kilogram
		Urine	Feces	Total excre-tion	Intake	Balance	
	kgm						grams
Von Wendt (36)	71	0 090	0 468	0 558	0 147	−0 411	0 0079
Sherman, Wheeler and Yates (31)	57	0 240	0 600	0 840	0 420	−0 420	
		0 330	0 360	0 690	0 450	−0 240	
		0 360	0 360	0 720	0 420	−0 300	
		0 390	0 270	0 660	0 420	−0 240	
		0 450	0 360	0 810	0 390	−0 420	
		0 390	0 450	0 840	0 390	−0 450	
		0 360	0 330	0 690	0 390	−0 300	
Average		0 360	0 390	0 750	0 411	−0 339	0 0132
Sherman and Winters (32)	54	0 180	0 450	0 630	0 240	−0 390	
		0 150	0 540	0 690	0 240	−0 450	
		0 180	0 540	0 720	0 240	−0 480	
		0 180	0 450	0 630	0 240	−0 390	
		0 270	0 570	0 840	0 600	−0 240	
		0 270	0 750	1 020	0 600	−0 420	
		0 200	0 750	0 950	0 600	−0 350	
		0 300	1 080	1 380	0 990	−0 390	
		0 240	1 020	1 260	0 990	−0 270	
Average		0 219	0 683	0 902	0 527	−0 375	0 0167
Sherman (6)	80	0 330	0 630	0 960	0 690	−0 270	
		0 510	0 450	0 960	0 630	−0 330	
		0 480	0 450	0 930	0 600	−0 330	
		0 540	0 480	1 020	0 630	−0 390	
		0 540	0 480	1 020	0 630	−0 390	
Average		0 480	0 498	0 978	0 636	−0 342	0 0122
Sherman, Wheeler, and Yates (31)	54	0 300	0 750	1 050	0 750	−0 300	
		0 390	0 570	0 960	0 780	−0 180	
		0 420	0 540	0 960	0 780	−0 180	
		0 390	0 570	0 960	0 750	−0 210	
		0 540	0 540	1 080	0 750	−0 330	
		0 570	0 570	1 140	0 750	−0 390	
		0 510	0 600	1 110	0 750	−0 360	
Average		0 446	0 591	1 037	0 759	−0 278	0 0192

TABLE 3—Continued

Author	Weight	Calcium in grams per 3-day period					Total calcium excretion per kilogram
		Urine	Feces	Total excre- tion	Intake	Balance	
	kgm.						grams
Rose (37)	45 5	0 174	0 594	0 768	0 846	+0 078	
		0 225	0 375	0 600	0 759	+0 159	
		0 243	0 651	0 894	0 747	−0 147	
Average		0 214	0 540	0 754	0 784	+0 030	0 0166
Sherman, Gillett, and Pope (30)	50	0 210	0 600	0 810	0 810	0 000	
		0 270	0 840	1 110	0 810	−0 300	
		0 300	0 840	1 140	0 840	−0 300	
		0 360	0 870	1 230	0 810	−0 420	
		0 360	0 810	1 170	0 810	−0 360	
		0 360	0 780	1 140	0 810	−0 330	
		0 360	0 870	1 230	0 840	−0 390	
Average		0 317	0 801	1 118	0 818	−0 300	0 0224
Sherman, Gillett, and Pope (30)	57	0 330	0 660	0 990	0 810	−0 180	
		0 410	0 630	1 040	0 810	−0 230	
		0 410	0 690	1 100	0 840	−0 260	
		0 370	0 450	0 820	0 840	+0 020	
		0 360	0 660	1 020	0 840	−0 180	
		0 370	0 480	0 850	0 840	−0 010	
		0 440	0 570	1 010	0 840	−0 170	
		0 340	0 570	0 910	0 840	−0 070	
Average		0 379	0 589	0 968	0 833	−0 135	0 0170
Sherman Winters and Phillips (33)	54	0 125	0 795	0 920	0 570	−0 350	
		0 285	1 065	1 350	0 930	−0 420	
		0 420	0 810	1 230	0 930	−0 300	
		0 330	0 930	1 260	0 930	−0 330	
Average		0 290	0 900	1 190	0 840	−0 350	0 0220
Sherman, Gillett and Pope (30)	52	0 320	0 840	1 160	0 900	−0 260	
		0 340	0 840	1 180	0 900	−0 280	
		0 390	0 810	1 200	0 870	−0 330	
		0 340	1 170	1 510	0 870	−0 640	
		0 270	0 870	1 140	0 870	−0 270	
		0 260	0 870	1 130	0 870	−0 260	
		0 250	0 570	0 820	0 900	+0 080	
		0 270	0 750	1 020	0 900	−0 120	
		0 260	0 900	1 160	0 900	−0 260	
		0 270	0 750	1 020	0 900	−0 120	
Average		0 29	0 837	1 134	0 888	−0 246	0 0218

TABLE 3—*Continued*

Author	Weight	Calcium in grams per 3-day period					Total calcium excretion per kilogram
		Urine	Feces	Total excretion	Intake	Balance	
	kgm						*grams*
Rose (37)	48	0 249	0 498	0 747	0 771	+0 024	
		0 186	0 480	0 666	0 963	+0 297	
		0 150	0 390	0 540	0 963	+0 423	
Average		0 195	0 456	0 651	0 899	+0 248	0 0136
Sherman, Winters, and Phillips (33)	67	0 180	1 170	1 350	0 720	−0 630	
		0 390	0 930	1 320	0 720	−0 600	
		0 315	0 840	1 155	0 720	−0 435	
		0 510	1 125	1 635	1 050	−0 585	
		0 420	1 050	1 470	1 050	−0 420	
		0 450	1 290	1 740	1 050	−0 690	
		0 405	1 050	1 455	1 050	−0 405	
Average		0 381	1 065	1 446	0 908	−0 538	0 0216
Sherman (6)	69	1 020	0 240	1 260	0 540	−0 720	
		0 840	0 330	1 170	1 170	0 000	
		1 080	0 670	1 750	1 200	−0 550	
Average		0 980	0 413	1 393	0 970	−0 423	0 0202
Rose (37)	56	0 210	0 792	1 002	1 149	+0 147	
		0 207	0 726	0 933	1 149	+0 216	
		0 207	0 573	0 780	1 149	+0 369	
		0 222	0 861	1 083	0 945	−0 138	
		0 231	0 567	0 798	0 945	+0 147	
		0 219	0 981	1 200	0 945	−0 255	
		0 216	0 660	0 876	0 945	+0 069	
		0 237	0 861	1 098	0 945	−0 153	
Average		0 218	0 753	0 971	1 021	+0 050	0 0173
Bogert and Kirkpatrick (12)	53	0 447	0 744	1 191	1 023	−0 168	0 0225
Rose (37)	54	0 243	0 870	1 113	1 149	+0 036	
		0 213	0 729	0 942	1 149	+0 207	
		0 207	0 645	0 852	1 149	+0 297	
		0 168	0 801	0 969	1 149	+0 180	
		0 099	0 399	0 498	0 945	+0 447	
		0 135	0 495	0 630	0 945	+0 315	
		0 228	0 708	0 936	0 945	+0 009	
		0 228	0 705	0 933	0 945	+0 012	
		0 180	0 717	0 897	0 945	+0 048	
Average		0 189	0 674	0 863	1 036	+0 173	0 0160

TABLE 3—*Concluded*

Author	Weight	Calcium in grams per 3-day period					Total calcium excretion per kilogram
		Urine	Feces	Total excretion	Intake	Balance	
	kgm						*grams*
Sherman (6)	61	0 450	1 260	1 710	1 380	−0 330	
		0 540	1 500	2 040	1 230	−0 810	
		0 600	0 780	1 380	1 230	−0 150	
		0 600	1 020	1 620	1 260	−0 360	
		0 600	0 960	1 560	0 960	−0 600	
		0 600	0 630	1 230	0 960	−0 270	
Average		0 565	1 025	1 590	1 170	−0 420	0 0260
Bogert and Kirkpatrick (12)	64	0 306	0 984	1 290	1 185	−0 105	0 0201
Bogert and Kirkpatrick (12)	70	0 393	1 152	1 545	1 185	−0 360	0 0221
Final average	56 6	0 343	0 710	1 053	0 831	−0 222	0 0185

tion is called to the fact that only one individual was in calcium balance and that individual for only one period   The group had an average intake of 0 33 gram per three days, an average output in the urine of 0 19 gram, in the feces of 0 60 gram, making a total average output of 0 79 gram resulting in an average negative calcium balance of 0 46 gram

In order to compare our results with other similar experiments taken from the literature, tables 3 and 4 and figure 3 have been constructed In table 3 are given figures for total calcium studies similar to ours, except that different food substances were used in many cases and that the calcium intakes were usually greater   The subjects here are arranged in order of increasing calcium intake rather than by weight We have recalculated the figures of these other investigators in order that they may be comparable to our figures, i e , all figures have been reduced to grams of calcium per individual per three day period   In table 4 are given the data for the calcium excretions of fasting men These observations differ from those in which the diet is inadequate in calcium only (v supra) because of the great drain on body tissues In figure 3 these data from the literature are given in graphic form for comparison with the values from figure 2, the average of which is

TABLE 4
Calcium studies on "fasting men" (taken from the literature)

Author	Weight	Calcium in grams per 3-day period					Total calcium excretion per kilogram	Negative nitrogen balance per 3 days
		Urine	Feces	Total excretion	Intake in water	Balance		
	kgm						*grams*	*grams*
Benedict (38)	58	1 020	0 000	1 020	0 000	−1 020		31 6
		1 086	0 000	1 086	0 000	−1 086		30 8
		0 950	0 000	0 950	0 000	−0 950		30 7
		0 913	0 000	0 913	0 000	−0 913		30 6
	52	0 957	0 000	0 957	0 000	−0 957		28 1
		0 998	0 000	0 998	0 000	−0 998		25 8
		0 914	0 000	0 914	0 000	−0 914		23 1
	49	0 718	0 000	0 718	0 000	−0 718		23 5
		0 611	0 000	0 611	0 000	−0 611		23 5
	47	0 568	0 000	0 568	0 000	−0 568		22 5
Average	52	0 873	0 000	0 873	0 000	−0 873	0 0163	27 0
Cathcart (39)	60	0 552	0 000	0 552	0 000	−0 552		31 0
		0 459	0 000	0 459	0 000	−0 459		25 0
		0 288	0 000	0 288	0 000	−0 288		25 0
Average	60	0 433	0 000	0 433	0 000	−0 433	0 0072	27 0
Mueller, Munk, Senator, and Zuntz (40)	57	0 885	0 208	1 093	0 171	−0 922		38 1
		1 070	0 208	1 278	0 171	−1 107		31 7
Average	57	0 977	0 208	1 185	0 171	−1 014	0 0208*	35 0
Mueller, Munk, Senator, and Zuntz (40)	60	0 305	0 094	0 399	0 219	−0 180	0 0067	33 2
Final average	58	0 769	0 031	0 800	0 035	−0 765	0 0137	28 4

* Weight before fast, used

TABLE 5

Summary chart

	Number of 3-day periods	Weight	Values for calcium in grams per 3-day period					Total calcium excretion per kilogram	Negative nitrogen balance per 3 days
			Intake	Urine	Feces	Total excretion	Balance		
		kgm						*grams*	*grams*
Average values of table 2	46	67 2	0 33	0 19	0 60	0 79	−0 46	0 0120	—
Average values of table 3	100	56 6	0 83	0 35	0 71	1 05	−0 22	0 0185	—
Average values of table 4	16	58	0 04	0 77	0 03	0 80	−0 76	0 0137	28 4

represented by the first column at the left in figure 3   A line separates the "fasting men" experiments from the others, as they are not quite comparable.   In figure 3, aside from the "fasting men," each of whose
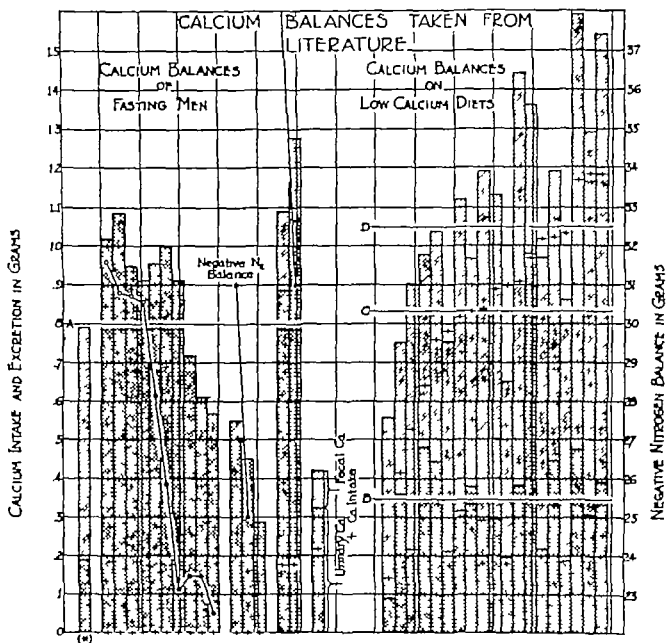

FIG 3 CALCIUM BALANCES TAKEN FROM LITERATURE

A, Average urinary excretion of "fasting men "   B, average urinary excretion of persons on a low calcium diet   C, average calcium intake   D, average total calcium output   Negative nitrogen balances in the 'fasting men" are represented thus o——o

* Graphic representation of average calcium balance from our control series

three-day periods are charted separately only the average value for each individual is charted

In table 5 are given the average values of tables 2 3, and 4   It

will be noted that the urinary calcium excretion is greater in the group from the literature than in our group   This is ascribed to the higher calcium intake   It has been stated that the urinary calcium increases on a low calcium diet (25)   This was probably due to some variation in the diet other than the calcium, such as the acid-base value (v supra)   This is borne out by the "fasting men" experiments, whose diet may be thought of as consisting of their own flesh, and therefore very acid   Their average negative nitrogen balance per three days of 28 4 grams would correspond to a calcium content of 0 067 gram (using Katz' figure for calcium value of human flesh) (26) Thus on this very low "intake" of calcium they had a very high average calcium excretion in the urine, 0 767 gram per three-day period

DISCUSSION

From an inspection of our data and the data collected from the literature it is seen that there is a certain very appreciable minimal requirement of calcium necessary to keep the body in calcium balance The literature gives abundant proof of this both by experiments on animals (8) (15) (27) (28) and by experiments on people (6) (29) (30) (31) (32) (33)   It is this negative calcium balance on a very low calcium intake which we wish especially to emphasize, and the varying degree of which under certain abnormal conditions we intend to make the subject of later papers   We believe that this is an aspect of calcium metabolism which can be quantitatively determined This appreciable excretion of a necessary body ingredient is not applicable to all body elements   Thus chlorine excretion during starvation sinks to almost zero and a diet deficient in chlorides does not lead to dechlorinization   Of direct interest in this connection are the experiments of Osborne and Mendel (27)   These investigators have shown that rats continue to gain weight on diets very low in magnesium, sodium, chlorine or potassium   However, when sodium and potassium were both very low or when either calcium or phosphorus was very low, the rats ceased to gain   Furthermore, an excess of magnesium was without avail in making up for the calcium deficiency It is their belief that sodium, potassium, magnesium and chlorine can be "husbanded" in the body, but that calcium and phosphorus and

at least one of the monovalent cations (sodium or potassium) must be furnished in certain minimal quantities Likewise Hamilton (28), from calcium balance studies on premature infants, concluded that a certain definite amount of calcium had to be excreted each day regardless of the intake and that only when calcium was furnished in excess of this amount was there a positive balance

The significance of this negative calcium balance on a low calcium diet can only be speculated on Hamilton (28) suggests that the calcium may be necessary to neutralize acids Osborne and Mendel (27) offer a similar explanation for the cessation of growth in their rats on diets very low in both sodium and potassium A second possibility suggests itself McCrudden (41) has shown that bone is constantly undergoing anabolism and catabolism Is it possible that the calcium liberated in the process of catabolism is not available for anabolism but must be excreted? The negative calcium balance on a zero calcium intake might then be thought of as an index of the endogenous calcium metabolism Experiments such as the ones now reported would thus be comparable to the early ones of Voit on nitrogen metabolism wherein the endogenous nitrogen metabolism was obtained by determining the nitrogen excretion during starvation (34) (39)

As a first step in the investigation of the cause of this negative calcium balance on a low calcium diet, it seemed of interest to see whether this relatively large amount of calcium ordinarily excreted on a very low calcium intake could be used during pregnancy to meet the fetal demands for calcium Therefore, a young woman was put on a low calcium diet for three three-day periods during the fifth and eighth months of pregnancy and six weeks after delivery by Caesarian section The findings are given in graphic form in figure 4 Some high calcium periods following the second and third group of low calcium intake periods are also charted, but will not be commented on here except to point out the rise in urinary calcium when the subject changed from a low calcium intake to a high calcium intake It will be noted that this subject excreted practically the same amount of calcium in all three groups of low calcium periods, regardless of whether she was supplying a small amount of calcium to the fetus as in the fifth month of pregnancy,

a large amount as in the eighth month of pregnancy, or none at
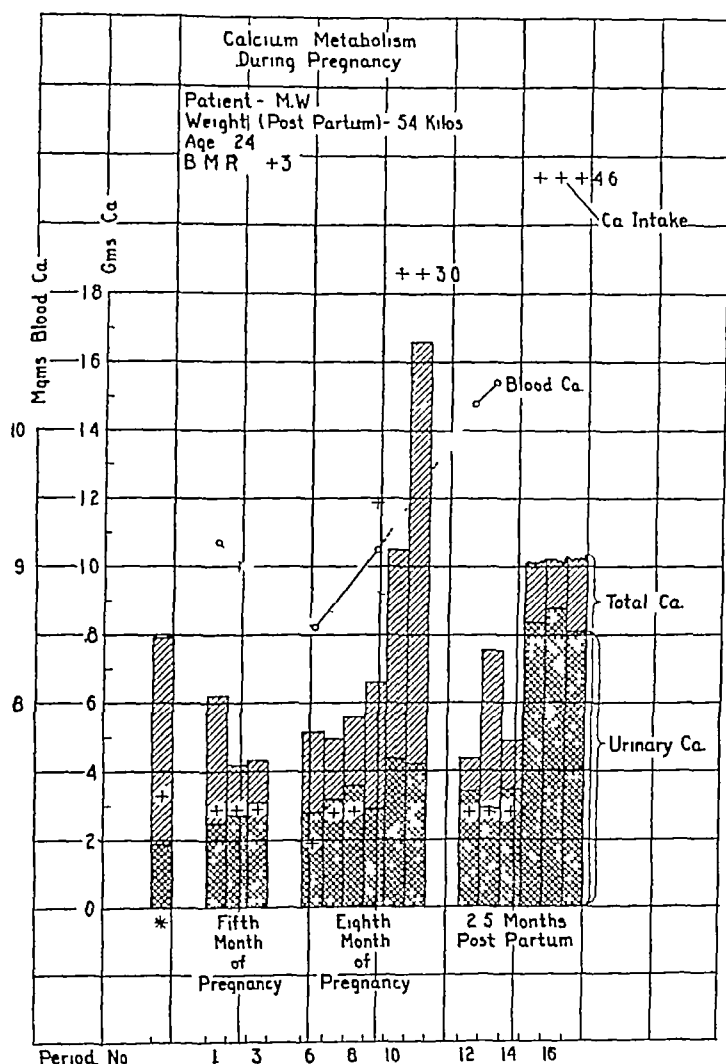all as during the second month after delivery, which was also one

FIG 4 CALCIUM METABOLISM DURING PREGNANCY
* Average calcium metabolism values of control series

month after cessation of lactation   Furthermore, during all three
periods of low calcium intake, this subject excreted about the same

amount of calcium as she would have been expected to do had she not been pregnant, as judged from the average excretion of our normal controls   Attention is called to the fact that this subject's non-pregnant weight is 54 kgm (average of controls 62 kgm) and that her sex is opposite to that of the controls

The serum calcium and phosphorus values of this patient are of interest   The serum calcium is confirmatory of the work of Widdows who found a low calcium level during pregnancy (35)

This experiment suggests that the calcium excreted on a low calcium diet is not available for the fetus, just as it is not available during growth for the building of bones (28)

SUMMARY

1 Figures showing the negative calcium balances in 46 three-day periods in thirteen normal individuals on a very low calcium intake are recorded

2 It is pointed out that before the negative calcium balance of two normal individuals on a low calcium diet can be compared, factors should be introduced to equalize differences in sex, age surface area, activity, acid-base properties of diet, values of other cations in diet, and phosphorus metabolism   It is further pointed out that unless two such individuals are in nitrogen equilibrium, further factors have to be introduced to offset the total acid value and the phosphoric acid content liberated during the destruction of the body protein

3 Our results are compared with similar but not identical experiments from the literature

4 It is emphasized that the negative calcium balance on a low calcium diet is an aspect of calcium metabolism which can be studied quantitatively under varying conditions

5 The cause of the appreciable negative calcium balance on a low calcium diet is discussed

6 Observations on a pregnant woman are included which tend to show that the calcium excreted on a low calcium diet is not available for the fetus   The calcium excretion during gestation is essentially normal

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STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

III THE EFFECTS OF THE THYROID HORMONE AND THYROID DISEASE[1]

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INTRODUCTION AND REVIEW OF THE LITERATURE

It is known that the internal secretion of the thyroid gland raises the basal rate of combustion of carbohydrate, fat and possibly protein, and consequently appreciably increases the total heat production of the organism, but its effect on calcium and phosphorus metabolism has not been definitely determined

Studies of the effect of thyroid secretion on the calcium metabolism have been reported in the literature, but they are inconclusive because unsatisfactory methods were employed and because the data obtained were scanty and contradictory   A short summary of these observations, however, is of interest in this discussion as a background for our experiments on the relation of the thyroid gland to calcium and phosphorus metabolism   As early as 1892, Koeppen (1) reported that there appeared to be a connection between exophthalmic goiter and osteomalacia and other bone diseases   Pierallini (2) in 1906 investigated the possibility of an intimate relation between thyroid function and the metabolism of calcium and phosphorus   In his experiments urinary calcium only was determined, the calcium intake was unknown and unrestricted because he assumed that the urinary content is an index of endogenous calcium metabolism   These assumptions are now known to be incorrect so that his results are of limited value   His figures showed the calcium and phosphorus ex-

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cretions to be normal in Basedow's disease   Another article published
in the same year by Scholz (3) gave the results of careful studies of the
metabolism of cretins before and after thyroid therapy   The un-
treated cretin was found to retain phosphorus and to excrete an abnor-
mally large quantity of alkaline earths   Administration of thyroid
preparations exerted no marked influence on the phosphorus metabo-
lism, but reduced the urinary excretion of the alkaline earths, partic-
ularly that of calcium   The fecal calcium, however, was increased
A year later, Silvestri and Tosatti (4) reported the effect of administra-
tion of thyroid extract on the calcium exchange in various diseases
They found that daily ingestion of "one tablet" of thyroid favored re-
tention of calcium   The calcium output was determined in one-day
periods during the first six days of thyroid medication, so that their
results can probably be explained by insufficient dosage of thyroid
and inadequate duration of observation   In referring to work done
with Bolaffio and Tedesco, Falta (5) in 1909 mentioned experiments
in which administration of thyroid increased the ratio of nitrogen to
phosphoric acid in the urine and increased the phosphate in the stools,
and concluded that there must be an increase in the fecal excretion of
calcium to account for the augmented phosphorus output in the stools
Another series of observations on the effect of thyroid feeding on cal-
cium excretion was made by Parhon (6) in 1912   In this very inter-
esting study the calcium exchange of nine rabbits was determined
both before and after administration of thyroid   The first group of
animals received 0 05 gram of thyroid daily and suffered a net loss of
0 007 gram of calcium per kilo per week, the second group of three,
all of which died within seventeen days, received 0 10 gram of thyroid
daily and lost 0 228 gram (average figures) of calcium per week, the
last three, which died within five days after the beginning of medica-
tion, received 0 3 gram of thyroid daily and lost on the average
0 662 gram of calcium per kilogram per week   These experiments
indicate that the thyroid does exert a stimulating influence on calcium
excretion   Kojima (7) in 1917 approached the problem from a dif-
ferent angle   He removed the thyroid gland and the parathyroid from
rats and then replaced the latter   He then determined the nitrogen
and calcium excretions and found that both had been diminished   He
could not, however, demonstrate in these experiments that adminis-

tion of thyroid affected the calcium metabolism. The report included no data concerning the adequate functioning of the parathyroid transplants and therefore the reduced calcium excretion after thyroidectomy might be explained by parathyroid deficiency

Kummer (8) in the same year published data obtained from observation for thirteen days of a patient with exophthalmic goiter Two and one half liters of milk were given daily, which meant that the calcium intake was approximately 5 grams per day No determinations were made of the basal metabolic rate and control calcium figures apparently were taken from the literature. The author concluded that the mineral excretion was high but very irregular, both the calcium and phosphorus losses being especially great. Because the quantity of calcium in the urine was normal and that in the feces was large, he attributed the high fecal calcium in Basedow's disease to a difficulty in absorption rather than an abnormal excretion

Vines (9) stated, without data, in his book on the relation of the parathyroid glands to disease, that calcium is lost from the body during diabetes mellitus and Graves' disease and retained by myxedematous individuals

In view of the undoubted importance of the thyroid secretion on calcium metabolism and the disagreement in the findings of previous authors, it seemed desirable to reinvestigate this subject.

EXPERIMENTS

We have studied the calcium phosphorus and nitrogen excretion in a series of patients with various thyroid diseases. The calcium excreted an individual while on a diet adequate in all respects except in calcium should represent chiefly the endogenous calcium metabolism. Our results demonstrate conclusively that increased thyroid secretion caused a striking accentuation of the calcium excretion and likewise increased phosphorus and nitrogen elimination.

The methods used have been described in a previous communication (10, The daily diets were practically identical for each individual and except for their very low calcium content were well balanced and adequate. There was however a temporary negative nitrogen balance in the patients suffering from exophthalmic goiter The low calcium diet has a two-fold value in that only permits a more ac-

curate evaluation of the endogenous calcium metabolism, but it also largely eliminates the unknown factor of unabsorbed calcium found in the feces

Serum calcium

In this series of 14 cases it was found that the serum calcium and phosphorus values remained within the accepted normal limits, though a few cases suggested a slight fall in these values during the period of observation   The serum findings gave no indication of the rate of calcium metabolism, as may be seen when the data on calcium and phosphorus excretion are examined

Calcium excretion

1 In exophthalmic goiter   Our normal standards for calcium excretion have been obtained from thirteen normal individuals who were maintained upon a similar diet inadequate in calcium   These data are to be found in Paper II of this series

The most obvious conclusions which can be drawn from our data are the marked increase of calcium excretion in exophthalmic goiter and hyperfunctioning adenomata of the thyroid, and the striking decrease found in myxedema   (See table 4 and chart 1)   This increase is out of proportion to the increase in the basal metabolic rate, for though the latter averages only 55 per cent above the normal in our six cases of exophthalmic goiter, the average calcium excretion is 170 per cent above our average control figures   These results are even more striking than they appear, for the average normal calcium excretion of 0 79 gram per three day period is based on the total excretion of men of average weight of 62 kgm , while the thyroid patients were thin individuals, some of them women, with an average weight of 50 kgm When calculated for three day periods, the average calcium excretion for the normal individual becomes 12 7 mgm   per kilogram of body weight, and for the patients with exophthalmic goiter 42 0 mgm   per kilogram of body weight, which is an increase of 231 per cent above normal   This increase in calcium excretion is shown most strikingly in the case of Norman G (chart 4), where 4 6 grams of calcium were

excreted in 3 days instead of 0 79 grams, the average found in the normal persons   Expressed in milligrams per kilogram of body weight, he excreted 96 4 mgm , which is 8 times the average excreted by our normal individuals on the same intake   This was out of all proportion to the change in his metabolic rate which was approximately
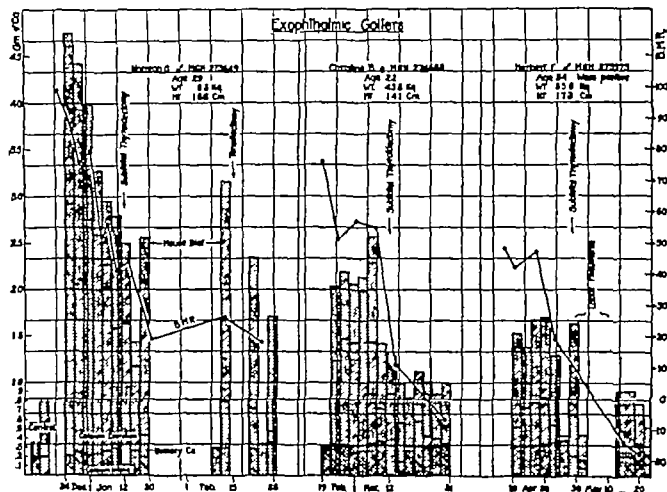

CHART 1   A GRAPHIC REPRESENTATION OF THE CALCIUM METABOLISM DATA
GIVEN IN TABLE 4, IN THE CASES OF NORMAN G , CHRISTINE B , AND
HERBERT F

The double cross hatching represents the calcium intake, the single cross hatch
ing up to the heavy dark line represents the urinary calcium excretion, the single
cross hatching above the heavy black line represents the fecal calcium excretion

twice normal   As his basal metabolic rate returned towards normal, the calcium excretion also approached normal   This was found to be true in all our other cases also   The calcium excretion in the *urine* alone was far higher than the *total* excretion found in our control cases   The increased calcium excretion in both urine and feces in thyrotoxic patients was about proportional   Because of the inade-

quate calcium intake on our routine diet, this increased calcium excretion must represent largely calcium loss from the body

2 *In toxic adenomata*   Added evidence of the effect of thyroid on calcium metabolism was furnished by two typical cases with hyperfunctioning thyroid adenomata in which the basal metabolic rates were not as high as in those suffering from exophthalmic goiter   The
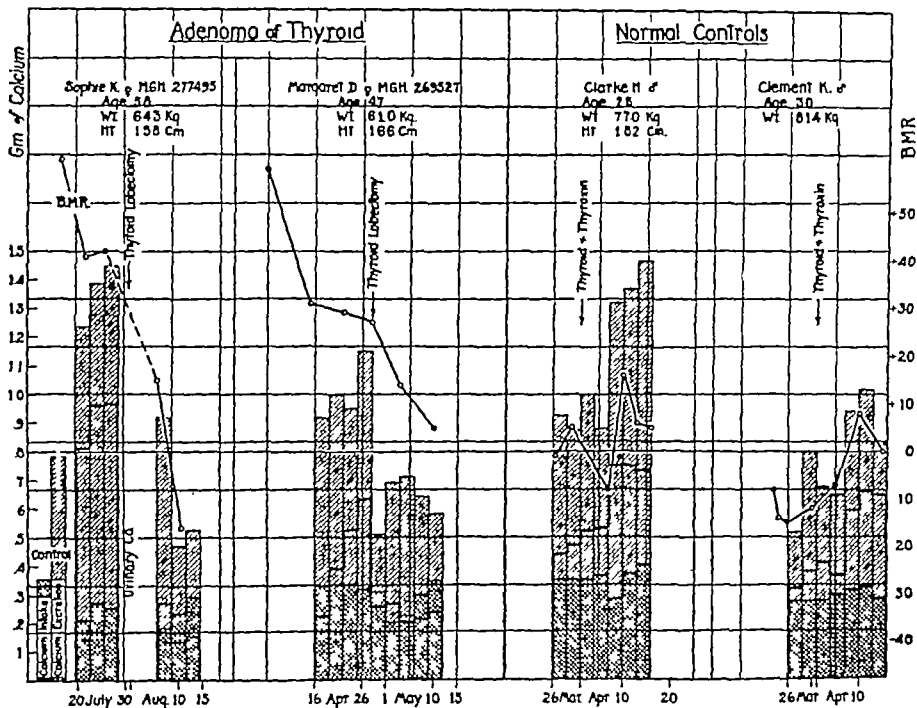

CHART 2   A GRAPHIC REPRESENTATION OF THE CALCIUM METABOLISM DATA
SHOWN IN TABLES 5 AND 7

The calcium intake, urinary calcium excretion and fecal calcium excretion are represented in the same manner as shown in chart 1

calcium, phosphorus and nitrogen excretions were likewise less elevated than in the series of patients with Graves' disease   (See table 5)   With this small number, however, it is impossible to say that there is any fundamental difference in the calcium excretion in the two diseases   The calcium excretion in these two cases was above normal and fell definitely after the adenomata were removed (chart 2)
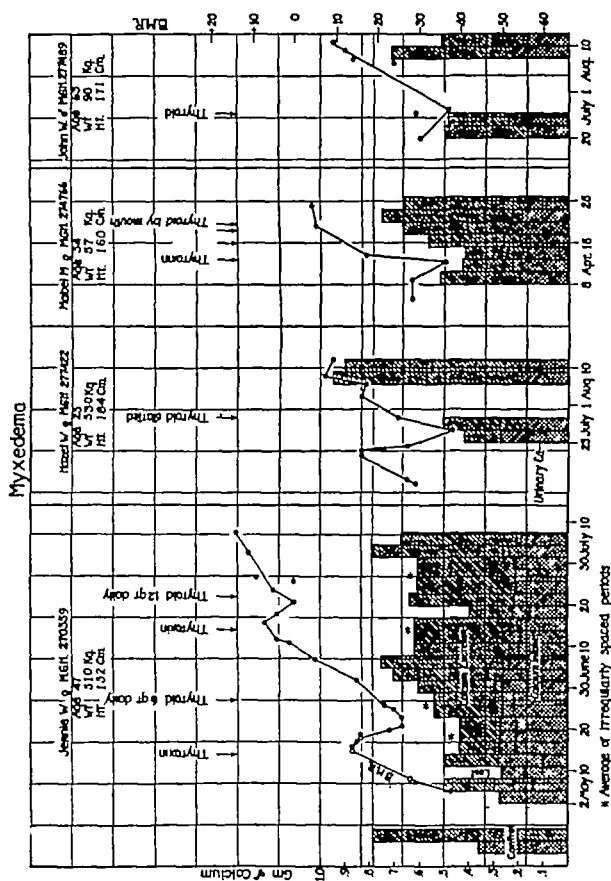
CHART 3   A GRAPHIC REPRESENTATION OF THE CALCIUM METABOLISM DATA GIVEN IN TABLE 6, IN THE CASES OF JENNIE W, HAZEL W, MABEL M, AND JOHN W

Here again the calcium intake, urinary calcium excretion and the fecal calcium excretion are represented as in chart 1

TABLE 2
Metabolic studies in cases with a high metabolism due to fever and leukemia

	Period number	Phosphorus Urine (gms)	Phosphorus Feces (gms)	Phosphorus Total (gms)	Phosphorus Intake (gms)	Calcium Urine (gms)	Calcium Feces (gms)	Calcium Total (gms)	Calcium Intake (gms)	Nitrogen Urine (gms)	Nitrogen Feces (gms)	Nitrogen Total (gms)	Nitrogen Intake (gms)	Total caloric intake	Blood serum Date	Blood serum Calcium (mgm per 100 cc)	Blood serum Phosphorus (mgm per 100 cc)	Basal metabolic rate (per cent)	Average temperature (rectal)	Diagnosis
Christian L.	I	1 307	1 070	2 377	1 759	0 348	0 492	0 840	0 323	36 32	2 7	39 02	26 5	5 353	October 25	8 2	3 1	+21	103	Lymphoblastoma
	II	1 392	1 099	2 491	2 146	0 386	0 420	0 806	0 350	30 23	4 0	34 23	28 9	5 910	October 29	8 02	3 01		102	Tertiary syphilis
	III	1 289	1 013	2 302	2 342	0 343	0 649	0 992	0 382	58 49	4 2	62 69	31 8	7 302	November 5	8 9	3 01			
	Average					0 359	0 520	0 879	0 351						November 12	8 2	5 3		102	
Myron G B	I	2 795	1 100	3 895	2 460	1 105	0 893	1 998	0 356	34 52	3 53	38 05	35 3	6 633	March 14			+37	98	Myelogenous leukemia
	II	2 448	1 143	3 591	2 478	1 053	0 673	1 726	0 366	67 00	3 55	70 55	35 5	6 693	March 16			+27	99	
	Average					1 079	0 783	1 862	0 361											
Charles A.	I	1 196	0 635	1 831	1 301	0 239	0 510	0 749	0 223	29 96	2 33	32 29	23 3	5 356	April 18			+19	102	Subacute bacterial endocarditis
	II	0 675	0 513	1 188	1 134	0 317	0 368	0 685	0 239	26 8	2 43	29 23	24 3	4 864	April 21	8 2	4 0	+33	102	Streptococcus viridans
	Average					0 278	0 439	0 717	0 231						April 25					
Jennie R.	I (2 day period)	1 520	0 430	1 950	1 240	0 305	0 248	0 553	0 161	23 85	2 16	26 01	21 6	2 831				+41	99	Myelogenous leukemia
	II	1 478	0 714	2 192	2 077	0 271	0 498	0 769	0 228	27 20	2 96	30 16	29 6	4 663						
	III	1 368	0 634	2 002	2 160	0 242	0 421	0 663	0 244	26 84	3 20	30 04	32 05	5 647						
	Average					0 272	0 389	0 661	0 211											

again that thyroid increases the endogenous calcium metabolism, for the extra elimination must come from the tissues, and presumably mostly from the bones   Similarly, two cases of parathyroid tetany with very low calcium levels in both their blood and excreta responded to thyroid therapy by a distinct elevation not only of their calcium excretion but also of their serum calcium level   This effect was prolonged and greatly benefited their general condition (12)

It is thus definitely established that increased thyroid secretion augments the excretion of calcium   This effect of thyroid secretion on calcium excretion is greater than its effect upon total metabolism. Thus the percentage variations from the normal controls, as shown in chart 4, indicate this marked difference, although the limits of variation of calcium metabolism are not as definitely established as those for the basal metabolic rate   The differences, however, exceed greatly the limits of variation in our normal series

The marked effect of thyroid activity in three women can well be compared (Elizabeth B and Christine B , who had Graves' disease, and Hazel W who had myxedema)   They were all young, and Elizabeth B and Hazel W were remarkably similar in age, height and weight   The thyroid effects are obvious from table 1

5 In other states of increased basal metabolism   Is the high calcium excretion of hyperthyroidism dependent on the increased thyroid secretion, or is it merely an accompaniment of an increased metabolism? This question could only be answered by determining the calcium excretion in cases with a high metabolism due to factors other than the thyroid, such as prolonged fever and leukemia   Three such cases— two suffering from subacute bacterial endocarditis, and one from lymphatic leukemia—showed a normal calcium excretion on our routine diet   A fourth case (M G B), suffering from myelogenous leukemia, showed an elevated calcium excretion approaching that found in hyperthyroidism   Three of the four cases, therefore, demonstrated normal calcium excretion in spite of an elevation of general metabolism.   The basal metabolic rates in these four cases varied from plus 21 to plus 41 per cent   We may conclude therefore that an increased calcium excretion is not necessarily a function of an elevated metabolic rate alone

X-rays of bones in exophthalmic goiter

It is not to be expected that all cases of hyperthyroidism would show osteoporosis of the bones because of this increased calcium elimina-

FIG 1   SINGLE X-RAY OF THE HANDS OF THREE WOMEN OF SIMILAR AGE, SIZE
AND WEIGHT

C is a normal control, *E* is the hand of a patient who is known to have had exophthalmic goiter for seventeen years, *M* is the hand of a case of severe myxedema

tion, which could probably be compensated for by a diet containing an adequate amount of calcium   In some cases of long duration, however, this increased calcium loss from the bones may be apparent in x-ray pictures   This is obvious in the x-ray pictured in figure 1, where

the hands of a patient with exophthalmic goiter of 17 years' duration, a case of myxedema, and a normal control are all shown in the same exposure   These individuals (all women) were approximately the same as regards age, weight and the shape of the hands   The marked
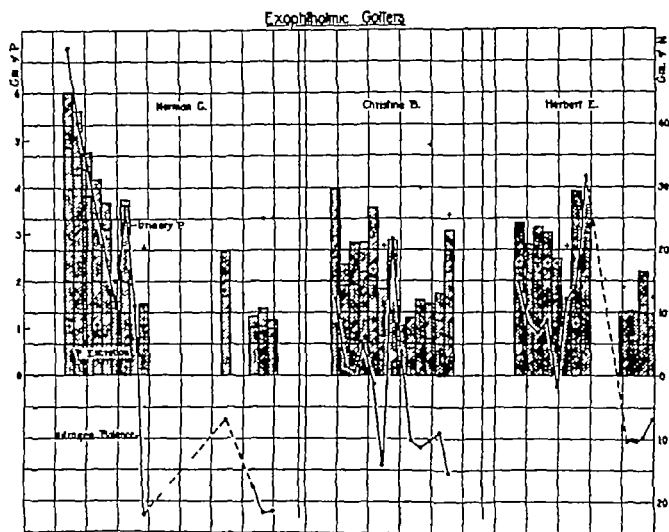
CHART 5   A GRAPHIC REPRESENTATION OF THE PHOSPHORUS METABOLISM DATA
      GIVEN IN TABLE 4 IN 3 CASES OF EXOPHTHALMIC GOITER

The phosphorus intake is represented by a cross, the heavy black line divides the fecal and urinary phosphorus excretions, the part of the column below being the urinary phosphorus excretion, and the part above being the fecal phosphorus excretion   The nitrogen balance is represented by o——————o, that above the zero line represents a negative nitrogen balance, and that below, a positive nitrogen balance

loss of calcium in this case of hyperthyroidism is very striking   We have seen one similar case, although less marked   This observation of osteoporosis in thyroid disease has recently been confirmed by Plummer (13)

Phosphorus metabolism

Phosphorus metabolism determinations were made in connection with those of calcium in all of these experiments   This gave us an opportunity to find out whether the calcium loss from the body as a result of thyrotoxicosis represents calcium phosphate withdrawn from the bones   If that were the case there should be a ratio such that the calcium loss to the phosphorus loss would equal that of calcium to phosphorus in tertiary calcium phosphate (Ca/P = 1 93)   In addition a correction should be introduced for the phosphorus liberated
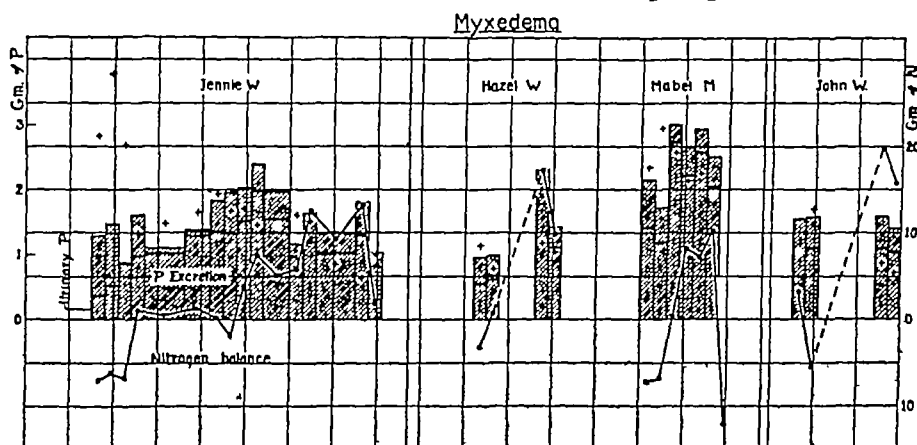
CHART 6   A GRAPHIC REPRESENTATION OF THE PHOSPHORUS METABOLISM DATA GIVEN IN TABLE 6 ON FOUR CASES OF MYXEDEMA

The phosphorus intake, urinary phosphorus excretion and fecal phosphorus excretion are represented as in chart 5

in protein metabolism (N/P = 17 4)   In table 3 there is included the actual phosphorus balance and the theoretical phosphorus balance calculated from the calcium and nitrogen balances as above described   In general the actual and theoretical balances agree as closely as could be expected   This supports the supposition that the increased calcium excretion represents, for the most part at any rate, calcium phosphate withdrawn from the bones   That some of this calcium may represent calcium carbonate withdrawn from the bones as suggested by Goto (14) is of course possible

TABLE 3

Phosphorus excretion

Period number	Phosphorus excretion		Phosphorus calculated from		Basal metabolic rate	Weight	Remarks
	Actual	Calculated	Endogenous calcium	Endogenous nitrogen			
Exophthalmic goiter							
	grams	*grams*	*grams*	*grams*	*per cent*	*kgm.*	
II	−4 99	−5 37	−2 37	−3 00	+86	56 3	
III	−4 52	−4 39	−2 08	−2 31	+76		Lugol's solution started
IV	−3 92	−3 81	−1 88	−1 93	+79		
V	−3 10	−3 01	−1 52	−1 49	+52	48 3	
VI	−2 50	−2 40	−1 34	−1 06	+56	48 3	
VII	−0 60	−1 88	−1 24	−0 64	+41	48 2	Subtotal thyroid ectomy
VIII	−3 55	−2 72	−1 19	−1 53			
IX	−0 06	−1 21	−0 58	−0 63	+44		Lugol's solution discontinued
X	+1 21	+0 19	−1 11	+1 30	+19	46 0	House diet for 3 weeks
XI							
XII	+0 285	−1 03	−1 43	+0 40	+26	48 0	Tonsillectomy House diet, for 1 week
XIII	+1 667	−0 35	−1 02	+0 67			
XIV	+1 925			+1 27	+18		
XV	+2 180	+0 57	−0 68	+1 25		48 3	
II	−0 61	−1 20	−0 73	−0 47	+70	49 0	
III	−2 59	−2 40	−0 65	−1 75			Lugol's solution started
IV	−1 15	−1 28	−0 40	−0 88	+40	46 7	
V	−1 13	−0 59	−0 59	0	+43	45 5	
VI	−0 95	−0 94	−0 77	−0 17			
VII	−0 71	−0 88	−0 57	−0 31	+16	46 5	
VIII	−0 06	+0 07	−0 33	+0 40			Subtotal thyroid ectomy
IX	+0 16	+0 30	−0 30	+0 60			
X	−1 16	−0 71	−0 29	−0 42			
XI	+0 01	−0 36	−0 30	−0 06			
XII	+1 06	+0 22	−0 32	+0 54	+7	43 9	
XIII	+1 32	+0 67	−0 12	+0 79			
XIV	+0 69	+0 15	−0 43	+0 58			
XV	+0 45	+0 39	−0 42	+0 81	+8	53 4	

Norman, G Age 29 (see Table 4)

Elizabeth, B Age 27 (see Table 4)

TABLE 3—*Continued*

Period number	Phosphorus excretion		Phosphorus calculated from		Basal metabolic rate	Weight	Remarks
	Actual	Calculated	Endogenous calcium	Endogenous nitrogen			
Normal controls							
	grams	*grams*	*grams*	*grams*	*per cent*	*kgm*	
Clark H Age 25 (see Table 7)							
I	−1 69	−1 08	−0 30	−0 78	0		
II	−0 04	−0 36	−0 27	−0 09			
III	−1 13	−0 80	−0 34	−0 46	−6		Thyroid started
IV	−1 83	−0 89	−0 27	−0 62			
V	−1 08	−2 00	−0 54	−1 46	+16		Thyroid stopped
VI	−0 85	−1 19	−0 51	−0 68			
VII	−4 03	−1 46	−0 55	−0 91	+48		
Clement I K Age 27 (see Table 7)							
I	−0 11	−0 43	−0 12	−0 31	−15		
II	−0 57	−0 60	−0 27	−0 33	−12		
III	−0 85	−0 62	−0 22	−0 40	−7		Thyroid started
IV	−1 13	−0 66	−0 18	−0 48			
V	−0 80	−0 81	−0 33	−0 48	+8		Thyroid stopped
VI	−1 34	−1 88	−0 36	−1 52			
VII	−0 68	−0 94	−0 29	−0 65	+2		
Myxedema							
Hazel B W Age 23 (see Table 6)							
I	+0 18	+0 09	−0 10	+0 19	−38	52 3	
II	+0 19	−0 20	−0 17	−0 03	−25		Thyroid, grs VI daily
III	−0 75	−0 52	−0 30	−0 22	−16	50 6	
IV	−1 12	−1 36	−0 36	−1 00	−7		
V	−0 40	−0 93	−0 35	−0 58	−9	50 7	

DISCUSSION

Thyroid secretion has been shown to increase markedly calcium excretion. The cause of this is as yet undetermined. There are several possibilities.

1 A stimulating effect on the parathyroid glands directly or through their sympathetic innervation. The absence of any marked rise in the serum calcium is very much against this, although Hunter and Aub (15) reported a case in which the administration of Collip's parathyroid extract increased the calcium excretion without appreciably rais-

ing the serum calcium level   Work now in progress will probably determine this possible relationship

2 A method of neutralizing the acid products of an increased metabolism   The neutralization of the phosphorus set free as a result of the increased protein metabolism might account for the increased calcium excretion   Such an explanation would be analogous to the increased calcium output following the injection of phosphates found by Greenwald (16)   However, unpublished observations (14) from this laboratory, on normal people following the administration of large amounts of acid phosphate, showed that a large part of this neutralization was accomplished by ammonia   The increased calcium excretion in these cases was not as marked as was observed in our cases of exophthalmic goiter   The other acid products resulting from an increased protein metabolism might call upon the calcium deposits in a similar manner   Studies on the total acid base metabolism in hyperthyroidism and myxedema are now being made and should help decide this point

3 The remaining explanation, namely a direct stimulating catabolic effect on the calcium deposits in the bones, seems the most likely one, and this would be in agreement with the general action of thyroid on other body tissues   It remains, however, for future work to establish this.

CONCLUSIONS

1 The calcium excretion in patients with exophthalmic goiter and in those with hyperfunctioning thyroid adenomata is increased markedly above the normal   This increase (231 per cent) is far greater than the increase in basal metabolic rate (55 per cent)

2 The ingestion of thyroid by normal individuals likewise increases the calcium elimination

3 The calcium excretion in myxedema is markedly diminished below that found in normal individuals

4 A marked increase in phosphorus excretion was also found   This increase was quantitatively such as to suggest that most of the calcium excreted came from tertiary calcium phosphate in the bones

5 This high rate of calcium elimination is not obvious in the blood,

TABLE 4

Exophthalmic goiter

Herbert L.   Age 34

Dates of period	Period number	Phosphorus Output Urine gm	Phosphorus Output Feces gm	Phosphorus Output Total gm	Phosphorus Intake gm	Calcium Intake gm	Calcium Output Urine gm	Calcium Output Feces gm	Calcium Output Total gm	Nitrogen Intake gm	Nitrogen Output Urine gm	Nitrogen Output Feces gm	Nitrogen Output Total gm	Nitrogen Intake gm	Total caloric intake	Blood serum Date	Blood serum Calcium mgm per 100 cc	Blood serum Phosphorus mgm per 100 cc	Basal metabolic rate Date	Basal metabolic rate Per cent	Weight Date	Weight kgm	Treatment and remarks
IV/10	I	2.28	0.96	3.24	2.40	0.40	0.53	0.99	1.52	0.32	33.9	7.4	41.3	26.5	8 215	IV/10	11.5	2.8	IV/7	+48	IV/10	56.2	
IV/13	II	2.06	0.74	2.80	2.02	0.32	0.73	0.66	1.39	0.32	29.8	8.5	38.3	29.9	8 516				IV/10	+42	IV/17	55.0	
IV/16	III	2.36	0.79	3.15	2.32	0.33	0.79	0.91	1.70	0.33	38.8	3.6	42.4	35.5	8 222				IV/17	+47	IV/22	54.8	
IV/18	IV	2.02	0.99	3.01	2.37	0.35	0.83	0.87	1.70	0.35	35.3	7.1	42.4	33.7	8 652	IV/22	9.6	2.8	IV/23	+19	IV/28	55.4	IV/20 Lugol's M ʒ t i d
IV/22	V	1.73	0.76	2.49	2.38	0.36	0.56	0.74	1.30	0.36	26.1	5.2	31.3	33.1	9 132								
IV/26	VI	1.66		2.75			0.43			0.38	19.5	3.5	23.0	35.3	9 632	IV/30	10.5	3.4					
IV/28	VII	2.74	1.18	3.92	1.11	0.21	0.65	0.99	1.64	0.21	26.8	10.1	36.9	12.6	3 471								IV/29, lobar pneum... until V/9
V/1	VIII	3.03	0.61	3.64	0.47	0.17	0.09	0.35	0.44	0.17	33.6	3.3	36.9	5.3	2 398								
V/11	IX	0.75	0.62	1.37	0.89	0.30	0.21	0.70	0.91	0.30	12.3	3.9	16.2	30.7	5 538	V/14	8.9	2.5	V/15	+14	V/17	52.0	V/16, Lugol's M ʒ t i d
V/16*	X	1.51	0.70	2.21	0.43	0.22	0.26	0.52	0.78	0.22	18.5	4.7	23.2	22.5	5 731				V/20	+18	V/21	53.6	
V/18*	XI	1.04	0.29	1.33	0.66	0.36	0.14	0.39	0.53	0.36	12.4	3.5	15.9	33.1	8 929	V/21	9.7	3.3	VI/12	+5			
V/21	XII					0.24				0.24	22.7				6 220								
V/23																							

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Norman G   Age 29

Date	Period											Date		Cal.			Date	Δ	Date	Wt.	Notes
VII/24	II	4 87	1 16	6 03	04	2 85	2 75	82 4	74	0 21	56 6	4 9 01 5	9 3	3 096	9 9	4 1	VII/23	VII/30 +99	VII/24	VII/24 56 9	VII/18, complete rest in bed, low calcium diet
VII/27	III	4 29	1 33	5 63	10	08	2 03	1 41	41	0 25	44 3	5 5 49 8	9 7	5 260	9 7	4 0	VII/28	I/2 +86	VII/27	VII/27 56 3	VII/27 began Lugol's M x t.i.d. VII/28, Lugol's M xx t.i.d.
VII/31	IV	3 84	0 93	4 77	85	2 75	1 92	27 0	98	0 22	36 5	6 2 47 7	9 1	4 114	9 1			VII/30 +79		I/3 48 3	
I/2	V	3 49	0 69	4 18	08	2 03	1 92	58 0	25	0 24	35 0	4 2 39 2	13 2	4 240	13 2			I/2 +52		I/4 47 8	I/4 Lugol's M x t.i.d.
I/5	VI	3 12	0 58	3 70	2 70	1 92	27 0	68	95	0 27	30 3	3 2 33 5	15 0	4 607	15 0	5 1	I/7	I/4 +56		I/8 48 2	
I/8	VII	1 59	0 44	2 03	1 81	58 1	20 2	78	0 29	20 5	3 7	3 27 8	16 6	5 365	16 6	6 0	I/8	I/7 +41		I/8 48 2	
I/13	VIII	3 62	0 13	3 75	19 1	64 0	86 2	50	0 12	30 1	1 0	4 30 5	3 8	1 783	3 8	4 2	I/10	I/13 +44		I/17 46 0	I/10 subtotal thyroidectomy
I/14	IX	0 79	0 29	1 08	02 1	18 0	251	43	0 27	22 2	1 1	2 3 123 3	12 3	4 254	12 3						I/13 Lugol's stopped
I/17	X	0 16	1 35	1 51	2 73	0 44	1 31	57	0 33	8 8	5 4 14 2	36 8	8 877	36 8		I/18	I/18 +19	I/17		I/17 to II/7 house diet
I/20	VI												9 0		9 0	3 8	I/18	I/18			
II/13	VII	1 68	0 95	2 63	2 92	80 2	363	16	0 30	23 1	8 7 31 8	38 8	8 559	38 8	3 8	I/5	I/23	II/8	II/8 48 0	I/27 tonsillitis, temperature 101.5
II/22	VIII	1 08	0 24	1 26	2 90	72 1	61 2	33	0 29	19 1	6 8 25 9	38 8	8 559	38 8	3 5	II/24	II/23 +18	II/27	II/27 48 3	II/13 tonsillectomy and adenoidectomy II/14 soft solid, high caloric diet (3000 calories) to II/19
II/25	IV	1 06	0 36	1 42	3 50	68			0 34	19 4	2 3 21 7	43 8	9 702	43 8			II/27 +16			II/20 low calcium diet
II/28	V	0 17	0 17	1 17	3 35	48 1	231	71	0 36	15 0	7 1 22 1	43 8	9 702	43 8						

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TABLE 4—Continued

Christine B Age 22

Dates of period	Period number	Phosphorus Output Urine (gm)	Phosphorus Output Feces (gm.)	Phosphorus Output Total (gm.)	Phosphorus Intake (gm)	Calcium Output Urine (gm.)	Calcium Output Feces (gm.)	Calcium Output Total (gm)	Calcium Intake (gm)	Nitrogen Output Urine (gm.)	Nitrogen Output Feces (gm.)	Nitrogen Output Total (gm)	Nitrogen Intake (gm)	Total caloric intake	Blood serum Date	Blood serum Calcium (mgm per 100 cc)	Blood serum Phosphorus (mgm per 100 cc)	Basal metabolic rate Date	Basal metabolic rate Per cent	Weight Date	Weight kgm	Treatment and remarks
II/21*	I	2 51	1 48	3 99		1 23	0 80	2 03	0 33	24 6	11 7	36 3	34 1	7 437				II/17	+76	II/19	42 9	II/19, transferred to Metabolism Ward complete rest in bed, low calcium diet
II/25	II	1 91	0 45	2 36		1 48	0 71	2 19	0 30	27 3	6 5	33 8	32 3	7 630	II/25	10 0	2 9	II/24	+51	II/24	43 5	
II/28	III	1 93	0 89	2 82		1 46	0 61	2 07	0 32	28 3	5 0	33 4	32 5	7 846				III/3	+57	III/3	42 9	II/28, 2 grams calcium lactate by mouth
III/3	IV	2 20	0 62	2 82		1 98	0 36	2 34	0 27	26 1	4 3	30 4	25 8	7 224								
III/6	V	2 09	1 50	3 59		1 47	1 09	2 56	0 35	28 5	11 2	39 7	31 0	8 253	III/8	9 5	4 3	III/10	+55	III/10	41 9	III/6 began Lugol's Mixt'd
III/10†	VI	0 84	1 00	1 84		0 72	0 69	1 41	0 37	13 0	6 4	19 4	33 6	9 127								
III/12††	VII	2 42	0 44	2 86		0 88	0 43	1 31	0 02	19 5	3 8	23 3	1 8	651	III/15	9 0	2 2	III/16	+11	III/16	41 9	III/12, subtotal thyroidectomy
III/16	VIII	0 71	0 35	1 06		0 57	0 42	0 99	0 16	8 0	2 1	10 1	6 9	3 221								
III/18	IX	0 59	0 62	1 21		0 54	0 31	0 85	0 27	7 2	3 3	10 5	20 8	6 345								
III/22	X	0 68	0 72	1 40		0 58	0 52	1 10	0 32	12 2	4 7	16 9	28 1	9 150								
III/24	XI	0 79	0 74	1 53		0 59	0 42	1 01	0 31	13 1	4 8	17 9	28 3	9 378								
III/27	XII	1 19	0 56	1 75		0 40	0 48	0 88	0 31	14 1	5 1	19 2	28 3	9 378								
III/30	XIII	2 21	0 88	3 09		0 66	1 00	1 66	0 32	11 9	0 9	12 8	28 3	9 124	III/29	9 3		III/31	−7	III/31	43 5	
IV/2	XIV	0 68	0 70	1 38		0 41	0 19	0 60	0 29	6 6	1 8	8 4	27 3	9 378	IV/2	9 5	2 5	IV/9	+19	IV/9	45 6	IV/2, Lugol's stopped
IV/3**																		IV/20	−2	IV/20	46 2	

* Period I is 4 days, reduced in the above table to the equivalent of 3 days.

** Period XIV is 1½ days results being multiplied by 2 to give equivalent 3-day amounts

† Period V Urine About 500 cc. may belong to period VI

†† Period VI Stool Contains 400 cc. of urine.

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Elizabeth B   Age 27

I/15	II	2 09	0 73	2 82	21 1	08 0	69 1	77	0 29	34 7	3 5 38 2	30 1	5 686	I/14	9 8		I/14 I/18	+79 +70	I/14 I/18	49 6 49 0	1/20 Lugol s M x t.l.d.
I/18	III	2 88	1 01	3 99	31 0	70 0	81 1	54	0 23	39	7 8 47 8	16 9	2 977			4 3					1/22 Lugol s M xv t.l.d.
I/21	IV	1 03	0 93	1 96	81 0	34 0	67 1	01	0 20	21 4	6 4 27 8	12 5	2 109								1/24 Lugol s M xx t.l.d.
I/24														1/24	10 2	4 7	1/26	+40	1/26	46 7	1/25 Lugol s discontinued. Rheumatic fever
II/1	V	1 68	0 93	2 61	48 0	77 0	68 1	45	0 27	15 7	6 5 22 1	22 2	8 665	II/5	8 9		II/1	+43	II/1	45 5	II/1 Lugol s M x t.l.d.
II/4	VI	1 88	0 03	5 11	56 1	28 0	54 1	82	0 28	21 7	4 7 26 4	23 5	7 048								
II/7	VII	1 28	0 82	2 10	39 0	81 0	591	40	0 26	20 1	5 4 25 5	20 1	6 881	II/8	8 9		II/8	+16	II/8	46 5	
II/10	VIII	0 91	0 75	1 66	80 0	35 0	60 0	95	0 29	13 3	3 8 11 4	21 3	7 595								
II/13	IX	0 73	0 78	1 51	67 0	33 0	471	00	0 30	10 8	3 5 14 3	24 8	7 485	II/12 II/13	8 9 / 8 9	2 4					II/11 Lugol s discontinued
II/16		0 97	0 32	1 29	14 0	37 0	29 0	66	0 32	8 0	2 5 10 5	3 2	872								II/13 operation subtotal thyroidectomy
II/19	J	0 51	0 24	0 73	76 0	30 0	62 0	91	0 31	11 0	3 9 14 9	13 8	4 013								II/15 Lugol s M v t.l.d.
II/23	VIII	0 35	0 41	0 76	80 0	23 0	73 0	96	0 32	8 6	7 6 16 5	25 6	6 853	II/20 II/22	6 4 / 9 6	3 2	II/23	+7	II/23	43 9	II/23 Lugol s M v t.l.d.
II/25	XIII	0 39	0 14	0 53	82 0	34 0	220	56	0 31	11 0	1 4 12 4	26 1	6 859								
II/28	XIV	0 32	0 57	0 89	58 0	51 0	671	18	0 33	12 6	2 9 15 5	25 5	6 819								II/28 Lugol s discontinued
III/3	XV	0 57	0 77	1 14	58 0	42 0	721	14	0 32	7 6	3 8 11 4	23 3	6 819	III/3	10 0		III/31	+8	III/31	53 4	

Rose Lee K   Age 63

IV/18	I	2 16	0 74	2 90	16 1	40 0	55 1	60	0 28	30 1	7 5 37 6	32 9	7 686	IV/16	10 8	3 0	IV/15	+57	IV/17	43 7	IV/23 Lugol s M xv t.l.d.
IV/21	II	0 89	0 89	2 70	40 1	15 0	79 1	94	0 21	28 9	2 43 1	23 6	6 367	IV/21	9 9	3 9	IV/21	+73	IV/27	42 2	
IV/24	III	1 91	0 83	2 76	46 1	35 0	66 2	01	0 21	27 9	3 83 1	23 6	6 432				IV/28	+62			
IV/28	IV	1 50	0 77	2 27	61 1	49 0	711	82	0 24	17 6	4 02 1	25 7	6 387	IV/30	9 9	3 3					V/5 Lugol s M xv t.l.d.
V/1	V	1 68	0 86	2 54	41 1	65 0	78 2	22	0 24	20 1	5 2 25 3	25 6	5 798				V/4	+57	V/6	41 8	V/6 Lugol s M xx t.l.d.
V/4																					V/6 operation
V/6	VI	1 86	0 54	2 40	61 1	97 0	61 2	40	0 22	23 5	3 2 28 7	24 1	5 951	V/6	9 9	3 0	V/6	Lugol s stopped	V/6	40 2	V/9 Lugol s stopped
V/12	VII	0 78	1 01	1 79	58 0	54 0	98 1	52	0 22	22 7	2 8 11 8	22 7	5 682	V/14	9 9		V/18	+27	V/11	40 2	V/17 Lugol s M v t.l.d.
V/15	VIII	0 72	0 76	1 48	78 0	61 0	59 1	20	0 25	24 8	4 3 17 1	24 8	6 499								
V/18	IX	0 25	0 73	0 03	97 0	55 0	53 1	09	0 23	16 4	3 0 10 9	23 6	6 991	V/21	9 0	5 7	V/21		V/21	42 6	
V/21	X	0 59	0 64	0 56	44 0	55 0	44 1	00	0 15	7 4	5 0 12 4	17 5	4 236								
V/23																					

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TABLE 4—Concluded

George R A   Age 24

Dates of period	Period number	\[P\] Urine (gm)	\[P\] Feces (gm)	\[P\] Total (gm)	\[P\] Intake (gm)	\[Ca\] Urine (gm)	\[Ca\] Feces (gm)	\[Ca\] Total (gm)	\[Ca\] Intake (gm)	\[N\] Urine (gm)	\[N\] Feces (gm)	\[N\] Total (gm)	\[N\] Intake (gm)	Total caloric intake	Blood serum Date	Blood serum Calcium (mgm per 100 cc)	Blood serum Phosphorus (mgm per 100 cc)	BMR Date	BMR Per cent	Weight Date	Weight Kgm	Treatment and remarks
IV/3	I	2 00	0 47	2 47	2 05	0 54	0 38	0 92	0 32	29 5	2 6	32 1	27 8	7 874				III/12	+58	III/12	56 5	III/30 transferred to Metabolism Ward, rest in bed low calcium diet
IV/6	II	1 92	0 92	2 84	2 05	0 72	0 62	1 34	0 32	28 2	2 7	30 9	27 8	7 874				III/29	+49	III/29	55 4	
IV/9	III	1 76	0 67	2 43	2 05	0 68	0 50	1 18	0 32	28 5	3 3	31 8	29 6	8 026	IV/5	10 5		IV/5	+31	IV/5	54 1	
																		IV/7	+23	IV/7	53 9	IV/10, Lugol's begun
																		IV/10	+15	IV/10	53 8	
IV/13	IV	1 81	1 04	2 85	1 91	0 67	1 02	1 69	0 36	28 6	6 4	35 0	33 2	8 632	IV/12	10 6						IV/15, transferred to Ward D
(IV/16)*	V	1 20	0 88	2 08	2 44	0 51	0 70	1 21	0 37	13 4	4 2	17 6	33 7	9 396				IV/23	+3	IV/28	52 9	IV/17, subtotal thyroidectomy, good recovery

* Period V, a 2-day period multiplied by 3/2

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TABLE 5

Hyperfunctioning adenomas of the thyroid

Margaret Catherine D   Age 47

Dates of period	Period number	Phosphorus Urine	Phosphorus Feces	Phosphorus Total	Phosphorus Intake	Calcium Urine	Calcium Feces	Calcium Total	Calcium Intake	Nitrogen Urine	Nitrogen Feces	Nitrogen Total	Nitrogen Intake	Total caloric intake	Blood serum Date	Blood serum Calcium	Blood serum Phosphorus	BMR Date	BMR Per cent	Weight Date	Weight Kgm	Treatment and remarks
IV/16	I	1.67	1.02	2.72	2.88	0.22	0.70	0.92	0.33	24.6	5.8	30.4	35.5	1551	IV/16	10.9	4.5	IV/5	+59	IV/16	62.6	
IV/19	II	2.03	1.08	3.12	2.84	0.39	0.61	1.00	0.34	29.9	6.4	36.3	39.7	1491	IV/23	10.1		IV/15	+31	IV/23	61.8	
IV/23	III	1.82	0.89	2.71	3.58	0.50	0.42			31.4	6.3	37.7	40.1	1210				IV/22	+29			
IV/25	IV	1.69	1.03	2.74	4.69	0.63	0.52	1.15	0.32	26.6	7.1	33.7	39.0	1306	IV/30	10.1	3.2	IV/28	+27	IV/28	62.0	IV/28 operation—lobectomy
V/1	V	2.28	0.44	2.72	3.13	0.25	0.42		0.81	25.4	4.4	29.8	0.9	714	V/7	9.6	2.5	V/3	+14			
V/4	VI	1.14	0.60	1.74	3.22	0.27	0.42	0.69	0.20	17.7	4.4	22.1	15.2	2918				V/10	+5			
V/7	VII	0.71	1.00	1.71	3.46		0.51		0.71	17.1	4.9	22.0	29.3	2460				V/25	+18	V/12	63.4	
V/10	VIII	0.46	0.85	1.34	2.95	0.21	0.43	0.64	0.30	13.0	5.5	18.5	29.7	2535								
V/13	IX	0.73	0.71	1.44	3.15	0.24	0.34	0.58	0.35	14.7	4.5	19.2	30.2	2537								

Sophie Jeanne K.   Age 58

Dates of period	Period number	Phosphorus Urine	Phosphorus Feces	Phosphorus Total	Phosphorus Intake	Calcium Urine	Calcium Feces	Calcium Total	Calcium Intake	Nitrogen Urine	Nitrogen Feces	Nitrogen Total	Nitrogen Intake	Total caloric intake	Blood serum Date	Blood serum Calcium	Blood serum Phosphorus	BMR Date	BMR Per cent	Weight Date	Weight Kgm	Treatment and remarks
VII/20	I	2.08	0.74	2.82	2.77	0.81	0.45	1.26	0.21	29.8	4.8	34.6	20.8	3977	VII/19	10.3	4.1	VII/3	+67	VII/17	64.1	Low calcium diet
VII/23	II	1.92	0.75	2.67		0.96	0.42	1.38	0.27	26.8	5.7	32.5	29.5	4823	VII/26	9.9	5.0	VII/13	+61	VII/22	62.3	
VII/26	III	1.72	0.60	2.32	3.31	0.97	0.48	1.45	0.68	31.5	3.9	35.4	20.8	3806	VII/27	10.0		VII/22	+41	VII/26	62.0	Lugol's M xv t.i.d. VII/31 operation—lobectomy VIII/3 Lugol's discontinued
VII/29	IV	0.89	1.00	1.89	3.90	0.27	0.65	0.92	0.18	12.9	2.2	15.1	16.3	3306				VII/26	+42			
VIII/5	V	0.81	0.93	1.74	1.30	0.13	0.35	0.48	0.21	10.7	7.6	18.3	16.3	3430				VIII/3	+15	VIII/3	60.7	
VIII/8	VI	0.49	0.57	1.06	2.10	0.15	0.37	0.52	0.29	9.7	7.6	17.3	21.7	4665				VIII/10	−16	VIII/10	61.5	
VIII/11																		IX/15	+4			

Blood serum: mg. per 100 cc.

Two-day period.   Results multiplied by 3/1.

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TABLE 6

Cases of myxedema

Susan F — Age 48

Dates of period	Period number	Phosphorus Output Urine	Phosphorus Output Feces	Phosphorus Total	Phosphorus Intake	Calcium Output Urine	Calcium Output Feces	Calcium Total	Calcium Intake	Nitrogen Output Urine	Nitrogen Output Feces	Nitrogen Total	Nitrogen Intake	Total caloric intake	Blood serum Date	Blood serum Calcium (mgm per 100 cc)	Blood serum Phosphorus (mgm per 100 cc)	Basal metabolic rate Date	Basal metabolic rate Per cent	Weight Date	Weight Kgm	Treatment and remarks
VI/3	I	0 80	0 88	1 68	0 52	0 07	0 61	0 68	0 14	9 3	8 1	17 4	7 6	1 921	VI/6	9 3	3 7	V/19	−33	VI/2	61 4	
VI/6	II	0 26	0 74	1 00	0 83	0 06	0 47	0 53	0 40	6 0	6 3	12 3	11 6	2 306				VI/2	−42	VI/7	63 0	
VI/9	III	1 78	0 41	2 19	1 36	0 20	0 44	0 64	1 03	16 1	1 7	17 8	10 8	2 248	VI/12	8 8	4 4	VI/5	−39			VI/7, thyroxin 10 mgm.
VI/12	IV	1 39	0 70	2 09	1 62	0 14	0 57	0 71	1 75	14 0	6 1	20 1	16 8	3 082				VI/9	−26			VI/10, thyroxin 10 mgm.
VI/15	V	1 17	0 80	1 97	2 10	0 10	0 67	0 77	1 85	24 2	4 5	28 7	12 6	3 611								VI/13, thyroxin 10 mgm.
VI/18	VI	1 34	0 65	2 99	1 56	0 12	0 66	0 78	1 28	29 8	4 8	34 6	18 5	3 438	VI/21	9 2	6 7	VI/17	+1	VI/16	58 5	
VI/21	VII	1 88	0 69	2 57	1 44	0 25	0 67	0 92	1 18	34 2	4 7	38 9	11 6	2 694	VI/26	10 3	8 8			VI/22	54 6	
VI/24															VI/28	11 3	7 6			VI/24	52 0	
VII/11																				VI/29	46 6	
VII/13	VIII	0 89	0 41	1 30	1 07	0 05	0 37	0 42	0 33	11 6			17 1	3 918	VII/14	8 3	5 4	VII/13	−5	VII/7	45 6	
																				VII/13	47 0	

Jennie W — Age 47

Dates of period	Period number	Phosphorus Output Urine	Phosphorus Output Feces	Phosphorus Total	Phosphorus Intake	Calcium Output Urine	Calcium Output Feces	Calcium Total	Calcium Intake	Nitrogen Output Urine	Nitrogen Output Feces	Nitrogen Total	Nitrogen Intake	Total caloric intake	Blood serum Date	Blood serum Calcium	Blood serum Phosphorus	Basal metabolic rate Date	Basal metabolic rate Per cent	Weight Date	Weight Kgm	Treatment and remarks
V/2	I	0 36	0 92	1 28	2 82	0 20	0 82	1 02	0 27	15 2	4 7	19 9	27 0	5 756	V/3	9 9		V/5	−38	V/6	53 0	
V/5	II	0 53	0 94	1 47	3 78	0 14	0 35	0 49	0 22	9 8	6 6	16 4	22 7	4 499				V/8	−28			
V/9	III	0 87	Lost	?	2 68	0 17	Lost		0 26	14 0			23 2	4 541	V/10	10 0	2 9					
V/11	IV	0 99	0 61	1 60	1 31	0 19	0 31	0 50	0 29	13 0	4 2	17 2	16 2	3 765				V/15	−14			
V/14									0 28				14 9	3 353	V/17	10 1	2 8	V/16	−14			V/14, thyroxin 10 mgm.
V/18	V*	1 01	0 42	1 09	1 51	0 21	0 22	0 43	0 28	15 3	3 2	18 5	20 1	4 639				V/17	−14			

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V/23	VI								0 28				21 9	5 275	V/24	10 0	3 5	V/18	−16			
																		V/19	−16			
																		V/20	−23			
																		V/21	−26			
																		V/23	−26			
																		V/25	−24	V/26	52 2	
																		V/26	−22			
V/27	VII†								0 22				23 0	5 199	V/26	9 5						V/27 6 grains thyroid q daily
		1 29	0 59	1 88	1 64	0 26	0 27	0 53		16 8	3 2	20 0										
V/29	VIII†								0 22				20 2	4 395						V/31	51 2	
VI/1	IX	1 36	0 46	1 82	1 93	0 29	0 32	0 62	0 26	20 3	4 6	24 9	25 0	5 658	VI/1	10 4	3 4	VI/2	−15			
VI/4	X	1 33	0 62	1 95	1 67	0 32	0 38	0 70	0 26	20 0	2 0	22 0	24 0	5 396								
VI/7	XI	1 51	0 52	2 03	1 70	0 23	0 52	0 75	0 26	24 1	3 7	27 8	24 0	5 469	VI/7	10 6	3 8	VI/7	−5	VI/8	50 6	
VI/10	XII	1 97	0 50	2 47	1 46	0 19	0 42	0 61	0 23	24 2	3 2	27 4	20 0	4 968	VI/12	10 1	3 5	VI/11	+1			
																		VI/12	+4			
VI/14	XIII†	1 56	0 40	1 96	1 45	0 18	0 43	0 61	0 26	22 2	3 0	25 2	24 0 15 8	5 469 3 822				VI/14 VI/15	±0 +11			VI/14 thyroxin 10 mgm.
VI/16	XIV†								0 20									VI/16	+7			
																		VI/17	+3			
																		VI/18	+4			
VI/19	XV	0 96	0 20	1 16	1 60	0 15	0 24	0 39	0 22	19 6	1 9	21 5	15 7	4 612	VI/19	10 1	3 4	VI/19	±0	VI/19	50 0	
																		VI/21	±0			
VI/22	XVI	1 19	0 43	1 62	1 51	0 17	0 47	0 64	0 22	22 0	3 5	25 5	13 0	4 217				VI/23	+5			VI/22 thyroid grains xii q. daily
																		VI/25	+1	VI/22	49 8	
VI/26	XVII**								0 20				11 8	3 597				VI/26	+9			
VI/29	XVIII	1 02	0 30	1 32	0 86	0 20	0 39	0 39	0 16	18 0	2 0	20 0	11 2	3 873						VI/29	48 2	
VII/1	XIX								0 22				9 4	3 847				VII/3	−10			
VII/4	XX	1 30	0 50	1 80	0 66	0 26	0 52	0 78	0 23	19 0	3 4	22 4	9 3	2 457				VII/6	−8			
VII/7	XXI	0 82	0 21	1 03	1 00	0 20	0 46	0 66	0 18	13 9	2 6	16 5	14 7	3 996	VII/8	10 7	3 9	VII/8	−14	VII/7	45 6	
																		VII/9	−13			
														VII/14	9 7	3 6	VII/10	−3				

Average of 2 uneven periods.
† Average of 2 uneven periods.
** Average of 3 uneven periods.

TABLE 6—Continued

Mabel M   Age 34

Dates of period	Period number	Phosphorus Output Urine (gm)	Feces (gm)	Total (gm)	Intake (gm)	Calcium Output Urine (gm)	Feces (gm)	Total (gm)	Intake (gm)	Nitrogen Output Urine (gm)	Feces (gm)	Total (gm)	Intake (gm)	Total caloric intake	Blood serum Date	Calcium (mgm per 100 cc)	Phosphorus (mgm per 100 cc)	Basal metabolic rate Date	Per cent	Weight Date	Kgm	Treatment and remarks
IV/11	I	1 41	0 74	2 15	2 36	0 07	0 45	0 52	0 20	18 9	4 0	22 9	30 1	5 589	IV/7	9 9	3 3	III/13	−29	IV/6	61 4	Previous basal metabolic r II/20/26, minus 17 per c II/25/26 minus 25 per cen
																		IV/8	−29			IV/7 low calcium diet
IV/15	II	1 18	0 53	1 71	2 97	0 13	0 32	0 45	0 19	19 6	3 9	23 5	30 4	5 954						IV/11	60 8	
IV/17	III	2 75	0 25	3 00	2 58	0 25	0 19	0 44	0 16	23 2	2 2	25 4	25 1	5 108	IV/14	10 6	3 2	IV/14	−36	IV/14	61 6	IV/14, thyroxin 10 mgm
IV/20	IV	2 22	0 43	2 65	2 13	0 27	0 29	0 56	0 20	31 9	3 5	38 4	30 3	5 878				IV/15	−17	IV/17	59 8	IV/18, thyroxin 5 mgm 1 m
IV/23	V	2 59	0 35	2 94	2 28	0 41	0 26	0 67	0 23	34 8	2 7	37 5	30 4	6 378	IV/22 IV/23	10 9	3 6	IV/22	−5			IV/21 thyroxin 5 mgm 1 m IV/23, thyroid grains iv i d
IV/27	VI†	2 02	0 50	2 52	1 90	0 34	0 42	0 76	0 19	31 3	4 4	35 7	25 6	5 323				IV/27	−4	IV/24	58 0	
IV/29	VII*	0 18			2 00	0 38	0 27	0 65	0 21	16 4	1 0	17 4	29 6	5 425				V/12	+13	IV/29	58 0	

* Period VII—Two days and a little over   Results multiplied by 3/2 for 3 day period

† Period VI—Lost one small stool and small amount of urine

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Hazel Bancroft W   Age 23

Notes:
- Low calcium diet started on 22nd
- VII/29 started thyroid grains vi q daily

VII/23											VII/14 −29 / VII/15 −27
VII/26 / VII/29	I	0 57 / 0 62	0 40.0 97 / 0 36.0 98	1 18.0 16 / 1 09.0 28	1 18.0 16	0 31.0 47 / 0 24.0 52	0 28 / 0 17	8 2 / 11 2	2 2 / 2 8	2 10 4 / 14 0	13 8 / 13 4	2 960 / 1 818	VII/22 9 9 4 0	VII/20 −16 / VII/21 −16 / VII/22 −26 / VII/24 −39 / VII/26 −38 / VII/29 −25	VII/14 52 3 / VII/15 53 3	VII/21 52 9
VII/7	III	1 64	0 46 2	10 1 29 0 49		0 34 0 83	0 22	20 1	3 2	23 3	19 4	2 609		VIII/3 −16	VIII/3	50 6
VIII/6 / VIII/9 / VIII/12	IV / V	1 78 / 1 10	0 52 2 30 / 0 33 1 43	1 12 0 57 / 1 07 0 55		0 39 0 96 / 0 35 0 90	0 23 / 0 20	29 6 / 21 3	4 8 34 4 / 3 1 24 4	17 1 / 14 6	3 236 / 3 348	VIII/12 10 7 4 4	VIII/6 −17 / VIII/9 −7 / VIII/12 −9	VIII/12 50 7

John Francis W   Age 63

Notes:
- Low calcium diet
- VII/31 thyroid grains vi q daily
- VIII/4 thyroid grains iv one day
- VIII/5 thyroid grains ix l. d.
- VIII/11 thyroid grains vi l.d.

VII/20 / VII/23	I	0 98	0 56 1 54	1 02 0 13		0 38 0 51	0 15	14 0	3 6 17 6	14 5	3 158	VII/19 9 5 4 0	VII/17 −26 / VII/20 −30 / VII/23 −37 / VII/24 −38 / VII/26 −29 / VII/29 −30	VII/17 91 7
VII/26	II	1 09	0 49 1 58	1 70 0 18		0 31 0 49	0 24	12 4	4 1 16 5	22 0	4 438	VII/26 9 7 3 1		
VII/7													VII/30 −25 / VIII/6 −14	
VIII/10 / VIII/13	III / IV	1 09 / 1 04	0 50 1 59 / 0 35 1 39	0 87 0 38 / 0 73 0 12		0 35 0 73 / 0 39 0 51	0 22 / 0 33	25 6 / 23 9	3 9 29 5 / 3 26 2	9 6 / 10 5	2 754 / 3 049	VIII/12 9 8 4 4	VIII/9 −12 / VIII/11 −9	VIII/11 86 8

Elizabeth Mary D   Age 52

Notes:
- Low calcium diet

		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	mgm. per 100 cc.	mgm. per 100 cc.		gm.
VI/5 / VI/7 / VI/10 / VI/13 / VI/16	I / II / III / IV	0 58 / 0 74 / 1 64 / 1 67	0 15 0 73 / 0 34 1 08 / 0 63 2 27 / 0 52 1 92	1 68 0 13 / 1 68 0 27 / 2 00 0 36 / 1 92 0 33		0 02 0 15 / 0 14 0 41 / 0 38 0 76 / 0 31 0 64	0 22 / 0 20 / 0 23 / 0 16	11 8 / 19 1 / 18 9 / 18 3	1 1 20 2 / 2 721 6 / 3 021 3	20 4 / 20 4 / 24 0 / 16 0	4 314 / 4 360 / 5 208 / 3 472	VI/5 10 5 2 7	VI/12 10 0 3 9 / VI/18 9 9	VI/4 −36 / VI/9 −40 / VI/16 −40 / VI/22 −39 / VI/26 −33 / VI/29 −39 / VI/30 −36	VI/7 76 0 / VI/10 76 2 / VI/13 75 2 / VI/16 74 9

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TABLE 7
Normal controls

Clark H Age 25

Dates of period	Period number	Phosphorus Urine gms	Phosphorus Feces gms	Phosphorus Total gms	Phosphorus Intake gms	Calcium Urine gms	Calcium Feces gms	Calcium Total gms	Calcium Intake gms	Nitrogen Urine gms	Nitrogen Feces gms	Nitrogen Total gms	Nitrogen Intake gms	Total caloric intake	Blood serum Date	Blood serum Calcium (mgm per 100 cc)	Blood serum Phosphorus (mgm per 100 cc)	Basal metabolic rate Date	Basal metabolic rate Per cent	Treatment and remarks
March 26	I	3 11	1 92	5 03	3 33	0 44	0 48	0 92	0 35	54 1			15 8	622	March 29	9 4	2 7	March 26	−1	March 24, low cal cum supper
March 29	II	2 45	0 92	3 37	3 33	0 47	0 41	0 88	0 35	43 1	4 0	47 1	15 8	622	March 31	8 1	2 8	March 30	−5	April 1, thyroid gr xss i d
April 1 / April 4	III	3 41	1 05	4 46	3 33	0 52	0 48	1 00	0 35	47 6	5 9	53 5	15 8	622	April 2	10 2	3 5			
April 7	IV	3 74	0 85	4 59	2 76	0 53	0 36	0 89	0 37	52 0	4 2	56 2	15 8	778				April 6	−8	April 7, thyroxin 10 mgm i.v April 9, thyroid stopped
April 10 / April 13	V / VI	3 98 / 3 44	0 92 / 0 95	4 90 / 4 38	2 82 / 3 53	0 75 / 0 75	0 57 / 0 61	1 32 / 1 36	0 28 / 0 38	56 1 / 56 4	5 3 / 3 5	61 4 / 59 9	16 / 19	939 / 420	April 12 / April 14	9 4 /	2 7 / 2 6	April 10	+16	
April 16	VII	6 25	1 31	7 56	3 53	0 73	0 74	1 47	0 39	56 7	7 2	63 9	19	420		10 6		April 16	+48	

Clement L K Age 27

Dates of period	Period number	Phosphorus Urine gms	Phosphorus Feces gms	Phosphorus Total gms	Phosphorus Intake gms	Calcium Urine gms	Calcium Feces gms	Calcium Total gms	Calcium Intake gms	Nitrogen Urine gms	Nitrogen Feces gms	Nitrogen Total gms	Nitrogen Intake gms	Total caloric intake	Blood serum Date	Blood serum Calcium	Blood serum Phosphorus	Basal metabolic rate Date	Basal metabolic rate Per cent	Treatment and remarks
March 26 / March 28	I	2 70	0 44	3 14	3 03	0 32	0 20	0 52	0 28	39 3	2 0	41 3	16 0	273	March 26	9 2	2 2	March 23	−8	March 23, a.m., low calcium diet.
															March 31	8 0	2 7	March 24	−14	March 31, thyroid gr vi i d
																		March 26	−15	
																		March 31	−12	

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														April 2	9 7	3 1	April 5	5	-7	April 5 thyroid xvid.
April 1	II	2 39	0 92	3 31	2 74	0 38	0 43	0 81	0 28	34 8	6 9	41 7	36 0|6 273							
April 5	III	3 05	0 54	3 59	2 74	0 41	0 25	0 67	0 28	39 1	3 9	43 0	36 0|6 273							
April 7	IV	3 24	1 01	4 25	3 12	0 37	0 25	0 65	0 30	44 3	3 7	48 0	39 6|8 520				April 10	10	+8	April 9 thyroid stopped
April 10	V	3 20	0 69	3 99	3 19	0 59	0 36	0 95	0 31	50 8	3 2	54 0	45 6|7 519							
April 13	VI	3 57	0 67	4 24	2 90	0 66	0 36	1 02	0 32	53 4	4 3	57 7	30 9|7 308	April 13	8 3	2 3	April 15	15	+2	
April 15	VII	2 89	0 33	3 22	2 54	0 65	0 19	0 84	0 29	49 8	2 3	52 1	40 5|6 687							

*Period IV urine: estimated 400 cc. lost on April 1

Urine period stops the morning after carmine has been given—9 a.m.

Feces period stops when carmine stops.

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for essentially normal values for calcium and phosphorus are found in the serum

6   X-ray evidence is presented which indicates that marked osteoporosis may develop in the bones from prolonged hyperthyroidism

7   The increased calcium excretion is not dependent upon the elevated metabolism alone, for three out of four cases with high metabolism due to leukemia or fever had normal calcium eliminations

PROTOCOLS

Norman G   No 273649   Age 29 years, white, male, married   Admitted December 17, 1925   Discharged   February 28, 1926

History   One brother had a goiter with hyperthyroidism   Patient has been married nine years   He has three children   One child died at age of 2 months   Nine months previous to entry patient noticed nervousness, palpitation, and tremor of extremities   Dyspnea and headache were present on exertion   There was a gradual progression of symptoms until four months previous to entry when swelling of neck and prominence of eyes was noticed with slightly increased sweating   One month previous to entry he had a sense of constriction in his throat and slight constipation   He has had nocturia for five or six months

Physical examination   He was a nervous, restless, sweating, flushed young man with slight prominence of the eyes   There was slight lid lag   There was a fine tremor of fingers and tongue   Tonsils were very large and cryptic   Thyroid was symmetrically enlarged, firm and smooth, with definite bruit   Heart showed no enlargement   There was a systolic murmur over the precordium   There was a palpable thrill at the apex   Skin was warm and moist

Laboratory findings   Urine was examined five times—there were a few red blood cells on three occasions and sugar was slightly positive on three occasions, otherwise urine was negative   Blood—red count was 4,450,000, white count 7100, hemoglobin 80 per cent   Differential count showed polymorphonuclears 45 per cent, small lymphocytes 48 per cent, large lymphocytes 6 per cent, and mast cells 1 per cent   Wassermann was negative   Non-protein nitrogen was 34 mgm per 100 cc   Phthalein excretion was 35 per cent   Basal metabolic rate on December 19, 1925 was plus 99 per cent with a weight of 48 3 kgm

Treatment and progress   He had complete rest in bed and after ten days, Lugol's solution was started   On January 8, 1926, he had a sub-total thyroidectomy with uneventful recovery   On January 26, 1926, he had an attack of acute tonsillitis with a fever of 104 5 degrees F   which lasted ten days   On February 3, 1926, he had a tonsillectomy and adenoidectomy performed

Impression   This was a most severe case of exophthalmic goiter, greatly improved but not cured by operation   This condition was complicated by severely infected tonsils

George R A No 275358 Age 24 years, white, male, married, truck driver
Admitted March 24, 1926 Discharged April 29, 1926

History For years he has had frequent frontal headaches For three years he has had shortness of breath and severe palpitation on exertion which has grad ually become more intense He also has had an occasional, non radiating, stab- bing precordial pain For one year he has noticed an increasing weakness, emotional instability, and a feeling of heat with marked sweating He had a markedly increased appetite but good digestion and regular bowels His neck was growing larger

Physical examination He was well developed and nourished Skin was moist There was tremor of the hands There were visible pulsations in his neck Thyroid was enlarged with marked bruit There were no eye signs There was a diastolic murmur heard at the left border of the sternum Blood pressure was 210/75 There was a capillary and corrigan pulse Heart rate was 130

Laboratory findings Urine and blood were normal.

Treatment and progress He had complete rest in bed and after two weeks Lugol's solution was started On April 17, 1926, a sub-total thyroidectomy was done He had an uneventful recovery Pathological report showed follicular hyperplasia

Impression This was a case of aortic regurgitation with a very mild Graves' disease Operaton was advised largely because of the heart lesion and it pro- duced a marked general improvement

Herbert B E No 275575 Age 34 years married, white, male, mail carrier
Admitted April 5, 1926 Discharged May 23, 1926

History He has been married ten years and has three children One child died at 5½ months from lobar pneumonia His wife has liver enlargement and some cardiac decompensation He had a Neisserian infection fifteen years ago For a year he has noticed prominence of his eyes Gradually he noted definite nervousness and irritability, excessive perspiration with a voracious appetite but loss of strength and 22 pounds in weight. For two weeks he has noticed a much enlarged thyroid with increased tremor of his hands, palpitation, and dyspnea on exertion He has had three transient attacks of precordial pain in the past month

Physical examination He was fairly well developed and nourished and in no distress He was very nervous Skin was moist and warm, with tan pigmenta tion There was marked bilateral exophthalmos and lid lag Thyroid was symmetrically enlarged There was lumbar lordosis Heart showed a forceful apex impulse but was not enlarged Sounds were rapid and regular Blood pressure was 175/75 There was a fine tremor of the hands

Laboratory findings Urine was normal Blood showed a red count of 4,680,000, white count 8,150 and hemoglobin of 75 per cent. Differential count showed polymorphonuclears 61 per cent, small lymphocytes 12 per cent, large lympho-

cytes 25 per cent, and eosinophiles 2 per cent   Non-protein nitrogen was 29 mgm per 100 cc   Wassermann was strongly positive on two examinations   Basal metabolism on April 7, 1926 was plus 48 per cent

Treatment and progress   He had complete rest in bed and Lugol's solution was started after two weeks   On April 29, 1926 a sub-total thyroidectomy was done Pathological report showed follicular hyperplasia   Following operation he had typical lobar pneumonia of the left lower lobe with a crisis on the eighth day Basal metabolic rate on May 20, 1926 was minus 18 per cent

Impression   Exophthalmic goiter, tertiary syphilis

Rose Lee K   No 275699   Age 63 years, female, white, married, housewife Admitted April 12, 1926   Discharged May 23, 1926

History   Patient has had fourteen children of whom seven died from obstetrical operations and three died in infancy   She has had no miscarriages   She has had recurrent tonsillitis   She also has had pleuritis and pneumonia   She had her menopause at 53 years of age

Present illness   She was always a hard worker   For the past year she had noticed increasing fatigue, nervousness, palpitation, and trembling of hands Swelling in her neck was noticed ten months previous to entry   Appetite became poor and she had epigastric distress relieved by food, and difficulty in swallowing For six months she has had shortness of breath and orthopnea   She was easily irritated and perspired easily   She has lost 57 pounds in weight

Physical examination   Patient was poorly developed and nourished, apprehensive, and nervous   There was slight exophthalmos, blepharitis, and slightly congested pharynx   Her thyroid was asymmetrically enlarged, nodular, and very firm   There was local glandular enlargement   Heart was enlarged to the left   Rate was rapid and regular   Liver edge was palpable   Blood pressure was 180/70

Laboratory findings   Urine was normal   Blood showed red count of 5,200,000, white count 15,000, and hemoglobin 70 per cent   Differential count showed polymorphonuclears 63 per cent, small lymphocytes 29 per cent, large lymphocytes 7 per cent, and mast cells 1 per cent   Non-protein nitrogen was 26 mgm per 100 cc   Wassermann was negative   Basal metabolic rate on April 15, 1926 was plus 87 per cent

Treatment and progress   She had complete rest in bed and after eleven days Lugol's solution was started   On May 6, 1926, a sub-total thyroidectomy was performed   Pathological report showed follicular hyperplasia   Basal metabolic rate on discharge was plus 27 per cent

Impression   Exophthalmic goiter

Christine B   No 274688   Age 22 years, female, white, married, hospital ward maid   Admitted February 15, 1926   Discharged April 3, 1926

History   She was separated from her husband   She had had no pregnancies She was strong and well up to present illness   She noticed a goiter one year

previous to entry and had been easily upset emotionally with crying and nervous spells   For one month she had noticed palpitation, slight prominence of her eyes, increased sweating, and slight difficulty in swallowing

Physical examination   She was a well developed and nourished, very short young woman who felt hot.   There was definite exophthalmos   There was a fine tremor of hands and tongue.   Thyroid was symmetrically enlarged, firm, with bruit   Heart was rapid and regular with no enlargement.

Laboratory findings   Urine was negative   Blood showed red count 5,656,000, white count 8,000, and hemoglobin 80 per cent   Smear showed polymorphonuclears 78 per cent, small lymphocytes 20 per cent, and large lymphocytes 2 per cent.   Non protein nitrogen was 32 mgm per 100 cc   Wassermann was negative. Basal metabolic rate on February 17, 1926 was plus 76 per cent, pulse 116, and weight 43 8 kgm

Treatment and progress   Patient had complete rest in bed and Lugol's solution was started at the end of two weeks.   On March 12, 1926, a subtotal thyroidectomy was done   Her basal metabolic rate at time of discharge was minus 7 per cent.   Patient was apparently cured by the operation

Impression   Severe exophthalmic goiter

Elizabeth B   No 274040   Age 27, female, white, single, stenographer Admitted January 9, 1926   Discharged March 3, 1926

History   Rheumatic fever at eight years for six months   She had two attacks of tonsillitis.   Catamenia was somewhat irregular   For ten years she had been nervous, marked by very easy excitability, crying and insomnia.   She had grown worse in the last three months and had lost 15 pounds in spite of an increased appetite   She had a feeling of warmth, fatigue, and palpitation   For ten days she had noticed swelling in her neck   She felt quite tired

Physical examination   Patient was nervous and twitchy   She was well developed but slightly thin with moist skin   Throat and tonsils were slightly red. There were no definite eye signs   Thyroid showed moderate, smooth, diffuse enlargement greater on the right with a marked bruit.   Heart was enlarged   A diastolic murmur was heard by one examiner only   Blood pressure was 144/80

Laboratory findings   Urine showed a few white cells, otherwise negative on five examinations   Blood red count 4,300,000, white count 8,000; hemoglobin 70 per cent.   Differential count showed polymorphonuclears 50 per cent, small lymphocytes 40 per cent and large lymphocytes 10 per cent   Wassermann was negative.   Non protein nitrogen was 29 mgm per 100 cc.   Basal metabolism on January 11, 1926, was plus 79 per cent.

Treatment and progress   Patient had complete rest in bed and Lugol's solution was started at the end of eleven days   During her stay in the hospital she had several attacks of severe pain in her joints accompanied by slight fever and a rise in her white blood count   Joints were all slightly swollen, warm, tender, but not red. She was relieved by salicylates.   February 13, 1926, a subtotal thyroidectomy was done   Pathological report showed follicular hyperplasia

Treatment and progress   She was greatly improved by the operation and left the hospital apparently well   Basal metabolism on February 23, 1926 was plus 7 per cent

Impression   Moderately severe exophthalmic goiter—complicated by a mild attack of rheumatic fever

Sophia J K   No 277495   Age 58 years, married, white, female   Admitted July 15, 1926   Discharged   August 8, 1926

History   Patient was in good health until three years ago when she first noticed a lump in the right side of her neck which had gradually increased in size   She then became nervous and "fidgety"   This increased so that two years ago her hands began to tremble, enough to bother her in writing   She also has noticed some palpitation and shortness of breath on the slightest exertion   There has been no increased perspiration   For the past three years she has had occasional stabbing pains in the lumbar region   During the past seven months she has had a vaginal discharge which is watery, at times bloody, and at other times purulent and bloody, no odor   She had measles and whooping cough as a child and rheumatism twenty years ago   Thirty years ago she was treated for metritis   Eleven years ago she had two uterine tumors removed

Physical examination   She was a nervous woman with marked coarse tremor of hands but no exophthalmos   There was a small round hard mass in the right lobe of the thyroid   There was a blowing systolic murmur at apex   Heart was normal in size   Blood pressure was 150/70   She had a moderate cystocele and a marked bloody discharge from the uterus

Treatment and progress   Operation was performed with excision of adenoma Dilatation and curettage was done, the curettings showing tuberculosis

Impression   Toxic adenoma of thyroid, tuberculous endometritis

Margaret D   No 269527   Age 48 years, white, married, female   Admitted April 8, 1926   Discharged   May, 13, 1926

History   She has been married twice   First husband divorced   She has one child living and well   Menopause occurred at 44 years   Patient entered the hospital one year previous to present entry complaining of nervousness of 8 months duration, some dizziness and dyspnea, increased perspiration, dislike of warm places, slight palpitation for one month, with a tight feeling in her throat   She had noticed enlargement of the thyroid for some time   She refused operation at that time   Two treatments by x-ray were without benefit and she feels she has grown worse since her first entrance   Dyspnea and weakness are increased She is emotionally unstable and she has noticed an increase in the size of her neck with some difficulty in swallowing

Physical examination   She was a well developed and fairly well nourished woman with considerable brownish pigmented areas of the skin   There was moderate exophthalmos and some tremor of fingers   The right lobe of the

thyroid had a solid, nodular enlargement, approximately 5 by 5 cm with a left lobe not appreciably enlarged   Heart showed no enlargement   There was a systolic murmur at the aortic area   Blood pressure was 124/80

Laboratory findings   Urine was negative   Blood was negative   Wassermann was negative   May 10, 1926, non protein nitrogen was 26 mgm per 100 cc

Treatment and progress   She had complete rest in bed and Lugol's solution was started after seven days   April 28 1926 a lobectomy was performed Pathological report ' Probably comes within limits of normal thyroid except for few miliary adenomata ''   She made an uneventful recovery   Patient was very quiet and calm when discharged   Basal metabolic rate on discharge was plus 5 per cent

Impression   Adenoma of thyroid with mild toxicity

John Francis W   No 277489   Age 63 years, widower   Admitted July 15, 1926   Discharged August 14, 1926

History   He dated his present pallor back twenty years   He said it came on following a "drinking bout" and had been present ever since   He thought his skin was more dry coarse, and yellow than formerly   Several years later he noticed his body hair (eyebrows, chest, axillary and pubic) began to fall out   For the past three years he had noticed he could not stand cold weather   He had had increasing weakness and inability to endure exercise   He was drowsy most of the time and said his memory was very poor for recent events   He had noticed increased constipation for the past few years   Appetite was fair   He had had puffy eyes of late   He could not obtain work as a collector because of his appearance There had been no increase in weight

Physical examination   He was a very obese man of 63 with a marked pallor of his mucous membranes   Skin had a yellow tint, was coarse, dry and appeared edematous—did not pit on pressure   His eyebrows were almost absent, as was the hair of his axillae chest and pubic region   His tongue was thick   Speech was slow and sluggish   There was a perforation of the nasal septum   Lungs showed chronic bronchitis   Heart was negative   Blood pressure was 160/74   Vessels showed moderate sclerosis

Laboratory findings   Urine blood and Wassermann were all negative   Non protein nitrogen on July 15, 1926 was 30 mgm per 100 cc

Progress and treatment   Patient was studied for 14 days before therapy was started   On July 31, 1926 he was started on thyroid extract grains 6 per day On thyroid therapy he was greatly improved   Basal metabolic rate on August 8, 1926 was minus 14 per cent

Impression   Myxedema, arteriosclerosis, emphysema and chronic bronchitis

Hazel B W   No 277422   Age 23 years, single, white, female   Admitted July 12 1926   Discharged August 12, 1926

History   For years patient had suffered from backache which was relieved by rest   For the past eight months she had had increasing general weakness often

accompanied by shortness of breath requiring rest after she had walked a few hundred yards   For the past six months she had been sensitive to cold, in fact she felt cold most of the time   She also had noticed she perspired very little and that her skin was dry and much coarser than previously   During this same period her memory had become poor   At times her tongue had felt swollen and tender With the above symptoms she had increased gradually in weight so that at the time of entry she weighed 130 as compared with her best previous weight of 102 Appetite was poor   She had been constipated for years   Catamenia had been absent for the past 2½ years

Physical examination   She was a young woman of 23, well developed and nourished   She appeared drowsy and apathetic   Face had a puffy appearance especially about the eyes   Skin was coarse and dry   Hair was coarse   She had a definite pallor   Blood pressure was 80/60

Laboratory findings   X-rays showed an enlarged sella turcica and sacroiliac arthritis   Electrocardiogram showed the T_2 to be 1 mm with small complexes and a rate of 85   Wassermann was negative   Blood—red count was 3,720,000, white count 7,500, hemoglobin 80 per cent   Non-protein nitrogen was 26 mgm per 100 cc   Phthalein excretion was 5 per cent.

Follow-up notes   September 11, 1926   She did not mind the cold   Her strength seemed to have returned   Appetite was better   She talked much faster Her skin was softer   She weighed 108 pounds   She was taking thyroid grains 6 daily   Basal metabolic rate was plus 35 per cent   Thyroid was cut down to grains 2 per day because of the high basal metabolic rate

November 16, 1926   Menses returned last month, lasted seven days, required three to four napkins per day   It was non-painful   Her basal metabolic rate was plus 1 per cent but she had some symptoms of myxedema returning   Thyroid was increased to grains 4½ per day

Impression   Myxedema

Elizabeth M D   No 276291   Age 52 years, white, married, female   First admission   May 13, 1926   Discharged   May 20, 1926   Second admission June 2, 1926   Discharged   July 2, 1926

History   She had her menopause seven years ago   She felt well until one year ago   At that time numbness and a cold feeling in her hands began   She gained 28 pounds in one year   She had minded the cold especially during the past winter   Her skin always had been dry and she had not perspired much   Her hands had become broad and puffy in the past two years   She was not excitable and her memory had become poor   There had been very little loss of hair   Her appetite was good   She had no dyspnea and no palpitation   She had recently stumbled over curbstones and for six months she had had difficulty in climbing stairs because of stiffness of the legs

Physical examination   She was a well developed and nourished, obese woman— dull and apathetic   Skin was coarse and dry   Hair was rather coarse   Heart

was not enlarged   The sounds were distant   There was a soft systolic murmur at the base   Abdomen was protuberant with diastasis recti   Extremities showed varicose veins and slight edema of both ankles

Laboratory findings   Urine was negative except for occasional white blood cells and epithelial cells on four examinations   Blood—red count was 4,300,000   White count was 6,400   Hemoglobin was 60 per cent.   Differential count showed polymorphonuclears 64 per cent, small lymphocytes 30 per cent and large lymphocytes 6 per cent.   Non-protein nitrogen was 38 mgm. per 100 cc.   Wassermann was negative   Basal metabolic rate was minus 34 per cent.   Weight was 73 5 kgm.

Treatment and progress   She had complete rest in bed and during this time metabolic studies were made   She was discharged on May 20th because of death in her family   No treatment was started

She returned, June 2, 1926   She was put on a low calcium diet and calcium metabolism studies were made before and during thyroid therapy

Impression   Myxedema.

Mable H M   No 274766   Age 34 years, white, married, female.   First admission February 19, 1926   Discharged February 25, 1926   Second admission April 6, 1926   Discharged April 28, 1926

History   She had been married eight years   She had one child.   She had had no miscarriages   For ten years she had been troubled with a dull ache in her lower back on bending forward and had felt tired   For five years this weakness and fatigue had become increasingly more severe   She noticed slowing of her mental reaction and a feeling of coldness   Catamenia was always irregular and painful   She was nervous, irritable, and slept poorly   Three and a half years ago her basal metabolic rate at the Homeopathic Hospital was minus 15 per cent and minus 18 per cent   For two years she had noticed thickness of her tongue and lips with difficulty in her speech   "Thyroid extract" at first resulted in headaches and palpitation but gave her relief

Physical examination   She was well developed and nourished, with thick lips and tongue   The skin of her face was slightly yellowish   Expression was slightly apathetic.   She had external hemorrhoids

Laboratory findings   Urine was negative.   Red blood count was 4,384,000, white count 8,600, hemoglobin 80 per cent   Differential count was normal.   Non protein nitrogen was 35 mgm. per 100 cc   Wassermann was negative   Basal metabolic rate on February 20, 1926, was minus 17 per cent.   Basal metabolic rate on April 8, 1926, was minus 29 per cent.   Fundi were negative.   X ray showed a large deep sella turcica.

Progress   This patient was very much improved by thyroid therapy but was still nervous and easily upset emotionally   She tired very easily and had frequent frontal headaches

Impression   Mild myxedema question of pituitary tumor· sacro iliac arthritis

Jennie W No 270359   Age 46 years, white, single, female   History of previous entry—June, 1925

History Two years ago she came to the Out Patient Department complaining of weakness, lack of ambition, loss of appetite and coldness of extremities   These symptoms had come on gradually over two years   Her speech was dull and list-less and she looked pale with pouches under her eyes   Menopause occurred six years ago   Basal metabolic rate on July 3, 1924, was minus 26 per cent   Basal metabolic rate on July 22, 1924, was minus 32 per cent   She was given thyroid extract—grains 1½ three times a day   Basal metabolic rate on August 11, 1924 was minus 13 per cent   Thyroid was increased to 7½ grains a day   Basal metabolic rate on September 5, 1924, was minus 1 per cent and patient felt better   Basal metabolic rate on January 26, 1925, was minus 24 per cent   Thyroid was reduced   Basal metabolic rate on April 10, 1925, was minus 13 per cent   She then entered the Hospital (June, 1925) and her condition was studied   Discharged   July 4, 1925

Second admission was on April 30, 1926   She returned because of recurrence of symptoms   After discharge she was taking 7½ grains of thyroid daily but her supply gave out about one month ago and symptoms slowly recurred   Basal metabolic rate on April 27, 1926, was minus 33 per cent

Physical examination She was well developed and nourished with slight puffiness under her eyes   There was moderate pallor of her skin with very slight dryness

Laboratory findings Urine was negative   Blood showed a red count of 4,216,000, white count 5,700, and a hemoglobin of 65 per cent   Differential count showed polymorphonuclears 38 per cent, small lymphocytes 47 per cent, large lymphocytes 4 per cent, mast cells 9 per cent and eosinophiles 2 per cent   Non-protein nitrogen was 23 mgm per 100 cc   Basal metabolic rate was minus 34 per cent   Wassermann was negative

Treatment and progress She had complete rest in bed   On May 14, 1926, she developed a definite psychosis with delusions of persecution   She wanted to leave the hospital   She was given thyroxin, 10 mgm intramuscularly   On May 16, 1926, her psychosis was much improved, 48 hours after 10 mgm of thyroxin intramuscularly, yet she retained certain ideas of persecution   The basal meta-bolic rate rose to plus 13 per cent on thyroid extract

Impression Myxedema complicated by mild psychosis

Susan B F No 265471   Age 48 years, white, married, female   Admitted June 1, 1926   Discharged July 15, 1926

In 1924 she was in the Massachusetts General Hospital with the diagnosis of (1) strangulated internal hernia, (2) myxedema, (3) chronic cystitis   A lapa-rotomy for intestinal obstruction was done

History She was born and had lived in Halifax, N S , until 7 years ago   She had nine children and no miscarriages   *Her periods stopped when her last child*

was born 17 years ago   She had not felt well since then   At that time she suf
fered from weakness and shortness of breath and often had to rest   She noticed
some paleness and bloating of her skin with considerable drowsiness   After two
years of these symptoms treatment with "pills and medicine ' relieved her and she
remained fairly well until 1918 when she had influenza, after which she became
weaker and more short of breath on exertion   No other symptoms occurred until
two or three years ago when a new group appeared   Her face became more
bloated lid fissures narrowed, at times her eyes almost closed and she had swelling
of her hands and ankles   Her skin was rough, pale, dry and flaked readily and in
creased thickness was especially noticeable over the extremities   Her hair fell out,
was coarser and more brittle   Her feet have been cold during the past three or
four years   At times she had tingling and numbness of her fingers and toes
Her speech was more indistinct, slower and her tongue felt thick   Her memory
was bad, she remembered past events better than present   She had frequent
dizzy spells   Her appetite was poor   She had been constipated for years
Occasionally she had short transient pains over precordium accompanied by
smothering and choking spells   All symptoms had grown progressively worse
so that she needed a three to four day rest in bed every week   Apparently she
had not taken the thyroid extract which had been prescribed for her

Physical examination   She was a pale, sluggish woman with well marked
edema about the eyes   Her skin was dry   Her hair was coarse and brittle
Tongue was large   Mucosae were pale   Heart was slightly enlarged to the left
Sounds were feeble and distant   There was a moderate amount of pitting edema
over the vertebral column from first lumbar vertebra down   Knee jerks were
extremely sluggish   There was a reddish brown scar of an old varicose ulcer, over
the middle anterior surface of the left tibia

Laboratory findings   Urine was negative except for occasional white blood cells
and hyaline casts on three examinations   Blood showed a red count of 3,088,000,
white count 3 160, hemoglobin 45 per cent. Differential count showed poly
morphonuclears 44 per cent, small lymphocytes 46 per cent large lymphocytes
1 per cent mast cells 4 per cent, basophiles 2 per cent, and eosinophiles 3 per
cent. Platelets were increased   Non protein nitrogen was 38 mgm per 100 cc
Basal metabolic rate was minus 35 per cent, weight 60 4 kgm

Treatment and progress   She had complete rest in bed

June 7, 1926   Patient was very weak   She complained of weakness, pains in
elbows and knees   She seemed semi stuporous at times   She had nausea and
vomiting   Patient was given 10 mgm thyroxin at 10 15 p m

June 10 1926   Patient had sore throat with fever   Thyroxin 10 mgm given

June 13, 1926   Patient had very low caloric intake and continued fever
She complained of pain in the muscles of arms and legs   Thyroxin 10 mgm again
given

June 22 1926   She was weaker and very nauseated   She ate nothing and
refused to coöperate

June 26, 1926   She seemed disoriented and irrational at times   Non-protein nitrogen 80 mgm per 100 cc   Serum phosphorus 8 8 mgm per 100 cc   White blood count was 4100   She was given 20 cc of CaCl₂ intravenously

June 28, 1926   She was given 20 cc CaCl₂ again and 500 cc 5 per cent dextrose intravenously   Non-protein nitrogen today was 110 mgm per 100 cc   At times she was irrational, at other times semi-comatose

July 14, 1926   She was much improved, was eating again, and did not vomit her food   She looked thin   The signs of myxedema had disappeared

Impression   The most marked type of myxedema

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STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

IV THE EFFECT OF THE PARATHYROID HORMONE[1]

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In our studies of calcium and phosphorus metabolism considerable
data have accumulated on the physiological effect of parathormone
(Collip's preparation) on normal and pathological subjects   We are
presenting in this paper such of these data as seem to us of value in
explaining its action

As explained in a previous paper (1) of this series, we employ a
diet inadequate in calcium when studying the effect of various
factors on the endogenous calcium metabolism   In a control series
of thirteen "normal ' male individuals with an average age of 41 years,
an average weight of 62 kgm , and an average calcium intake of 0 33
gram per three-day period, there was an average output in the urine
of 0 19 gram, in the feces of 0 60 gram, making a total average output
of 0 79 gram, and an average negative calcium balance per three day
period of 0 46 gram (1)   In the observations about to be described we
have studied the quantitative effect of parathormone injection on this
negative calcium balance and have as controls both the series sum
marized above and periods without medication on each individual
subject   The methods employed in the preparation of the diet, the
collection of the excreta, and the chemical analysis of the material
have been described in a previous paper (2)

Case I   This case is presented first because it is in every way
characteristic and because it presents the minimum number of

complicating factors   The subject was a girl of 16 years whose sole abnormality was otosclerosis of serveral years duration   We have no reason to suppose that otosclerosis affects the action of parathormone, although it may have some quantitative effect on the negative calcium balance while the subject is on a low calcium diet (3)   She was studied for twelve three-day periods on a low calcium diet   There was a three-day period on low calcium diet before the investigation was started, and this practice was adhered to in the other investigations reported in this paper   She received parathormone injections from the third to the eighth periods inclusive   In chart Ia and Ib are given the data of the calcium, phosphorus, and nitrogen balances, and of the blood serum calcium and phosphorus

From the data the following observations of the effect of parathormone upon a subject receiving a low calcium diet are noted

(i) During parathormone administration there is an increase in the negative calcium balance (in this case 165 per cent)

(ii) Following cessation of parathormone administration, the negative calcium balance is less than in the control periods, suggesting a compensatory mechanism

(iii) The calcium excretion in the feces is unaffected by parathormone administration

(iv) During parathormone administration the serum calcium rises   The increased urinary calcium excretion is roughly parallel to this rise

(v) Both the rise in the urinary calcium on administration of parathormone and the fall in the urinary calcium on cessation of parathormone tend to be gradual   (The rise is unusually abrupt in this case)

THE PHOSPHORUS METABOLISM

The calcium losses from the body are at the expense of the calcium deposited in the bones   This calcium is largely deposited as tertiary calcium phosphate (Ca P 1 93 1), but also partly as calcium carbonate, so that the ratio of calcium to phosphorus in bone is approximately 2 23 1   Therefore, it is of interest to determine whether phosphorus excretion during parathormone injection corresponds to the increased calcium excretion   If such were the case and all other fac-

tors were constant, the ratio of the negative calcium balance to the negative phosphorus balance should be 1 93 1 or 2 23 1, depending on whether parathormone affects only the calcium in calcium phosphate or whether it affects both the calcium in calcium phosphate and the calcium in calcium carbonate   As the difference between these two factors is less than the limit of error of our data, we have arbitrarily chosen the Ca P factor as 2 23 1   Before a theoretical phosphorus balance thus obtained can be compared with the actual phosphorus balance, a correction has to be made for the phosphorus involved in the deposition or liberation of protein (N P = 17 4)   Therefore, the phosphorus equivalent of the nitrogen balance and the phosphorus equivalent of the calcium balance have been calculated   The sum of these equals the "theoretical phosphorus balance," it being assumed that the phosphorus in muscle, body fluids, etc , would tend to remain in equilibrium   When we later have occasion to use the expression "theoretical phosphorus excretion" we mean the phosphorus intake plus the "theoretical phosphorus balance "   It should be noted that since the phosphorus intake is relatively high and since there must be considerable error in its estimation, the determined phosphorus balance is apt to vary from the theoretical by a certain fixed amount Since the diet is constant throughout the experiment, this error will be constant   In comparing the "theoretical phosphorus balance" with the actual phosphorus balance, the relative changes from period to period are therefore more significant than the actual difference in the two balances in any one period   In order to study these balances, we have constructed chart Ic.   Here the actual and theoretical phosphorus balances have been recorded

From an inspection of the phosphorus data (chart Ib), we have the additional observations

(vi) The urinary phosphorus is increased by parathormone administration without an effect upon the nitrogen balance

(vii) The fecal phosphorus is little if at all affected by parathormone injection

(viii) The serum phosphorus is decreased during parathormone administration

(ix) As the serum phosphorus falls during parathormone administration, the serum calcium rises
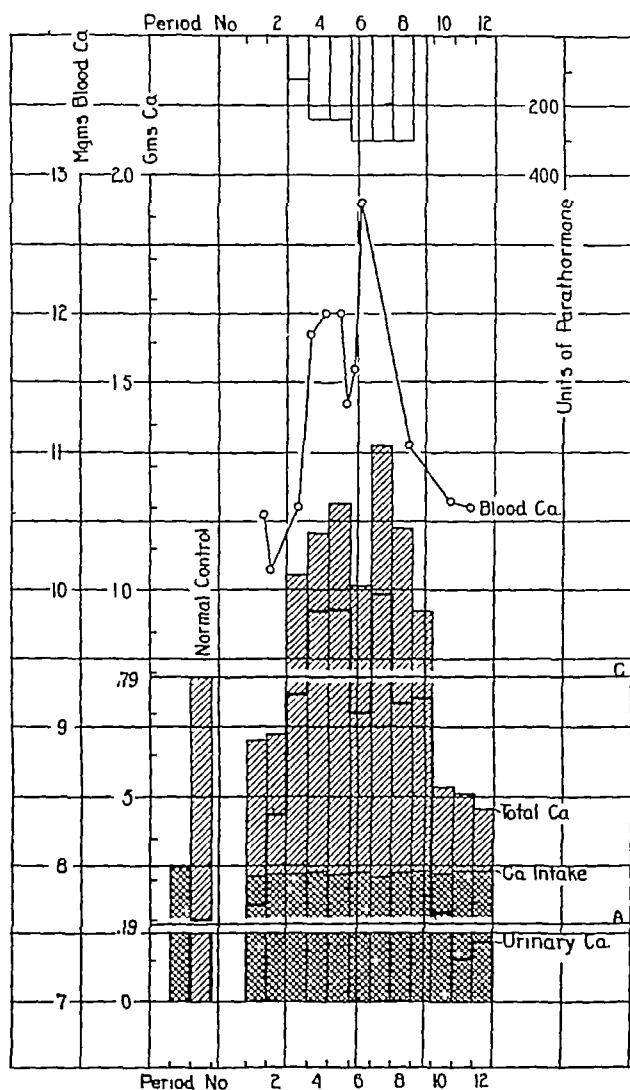
CHART Ia   CALCIUM METABOLISM IN CASE I

Line *C* represents average total calcium excretion of control series on a low calcium diet   Line *A* represents average urinary calcium excretion of control series on a low calcium diet (*1*)   These lines have the same significance in later charts

(x) The urinary phosphorus excretion tends to rise abruptly, often to its highest point during the first period of parathormone administration, and to fall abruptly to the pre parathormone level in
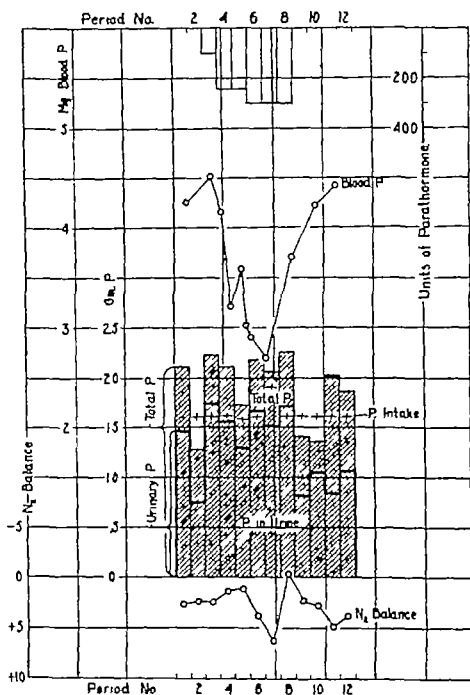
CHART Ib PHOSPHORUS METABOLISM IN CASE I

the first period following cessation of parathormone   This is in contrast with the urinary calcium excretion which shows a latent period

A careful examination of chart Ic shows a relationship between the theoretical and calculated phosphorus balances   This seems hit or miss on first inspection but will be found constant in all later experi

ments, namely, that there is *a phosphorus excretion in excess of the theo-retical amount during the first period or periods of parathormone admin-istration, and a phosphorus excretion less than the theoretical amount during the first period or periods following cessation of parathormone*
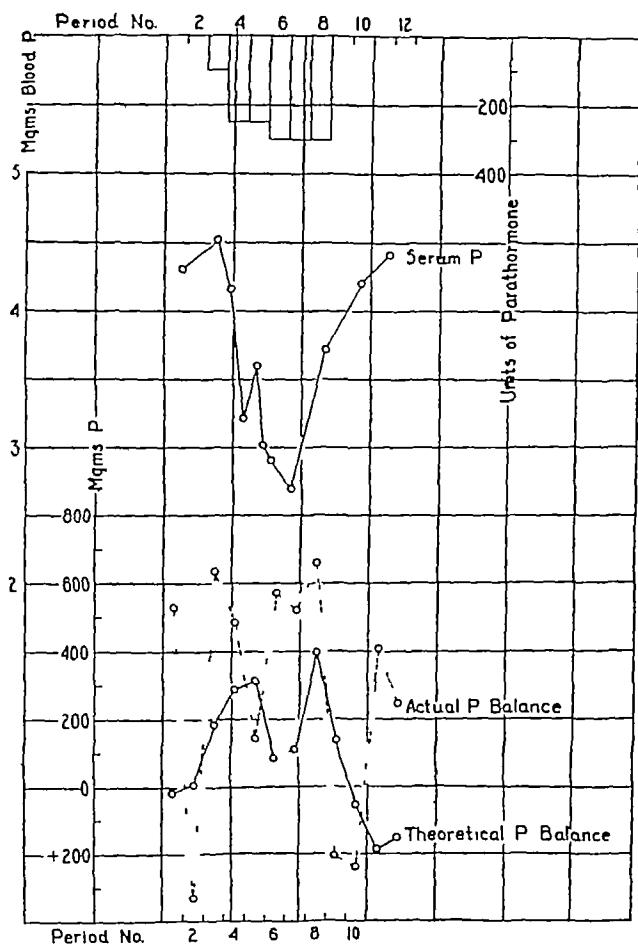
CHART IC "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN CASE I

One can predict variations in the urinary calcium excretion for any given period from chart Ic by the relationship of the theoretical phos-phorus balance to the calculated phosphorus balance of the preceding period   Thus periods 3 and 6, where the actual phosphorus excre-

tions rise much more than the theoretical phosphorus excretions, are followed by a rise in the urinary calcium in periods 4 and 7, periods 5 and 9, where the actual phosphorus excretions fall much more than the theoretical phosphorus excretions, are followed by a fall in the urinary calcium in periods 6 and 10

It will now be necessary to consider the significance of an actual phosphorus excretion in excess of a theoretical phosphorus excretion One of two explanations seems likely   Possibly when calcium phosphate is mobilized from the bones the phosphorus is excreted more rapidly than the calcium   Evidence against this explanation is found in case VI of this paper   Or possibly it may be due to an excretion of the phosphorus in solution in body fluids, blood, etc.   If such is the case one would expect the serum phosphorus to vary with the discrepancy between the actual and theoretical phosphorus excretions   In chart Ic, it will be noted that this appears true   We have then these further observations

(xi) During the first period of parathormone administration more phosphorus is excreted than can be explained by the calcium and nitrogen balances (theoretical phosphorus excretion)

(xii) During the first period following cessation of parathormone administration, less phosphorus is excreted than can be explained by the calcium and nitrogen balances

(xiii) During parathormone administration any tendency for the actual phosphorus excretion to rise above the theoretical phosphorus excretion is followed by a tendency for the urinary calcium excretion to rise after a latent period of about 3 days (1 period)   The converse is likewise true

(xiv) A rise in the actual phosphorus excretion above the theoretical phosphorus excretion is apparently partly at the expense of the phosphorus in the body fluids and results in a fall of the serum phosphorus

From case I we have made certain observations   These are confirmed by the other cases and new observations are added

Case II   This case is very similar to case I.   The subject was a boy of 16 who had symptoms of otosclerosis for about four years He was studied for thirteen three-day periods on a low calcium diet, then sent home for three months on a normal diet, and finally further studied on a low calcium diet for fifteen more three-day periods   The
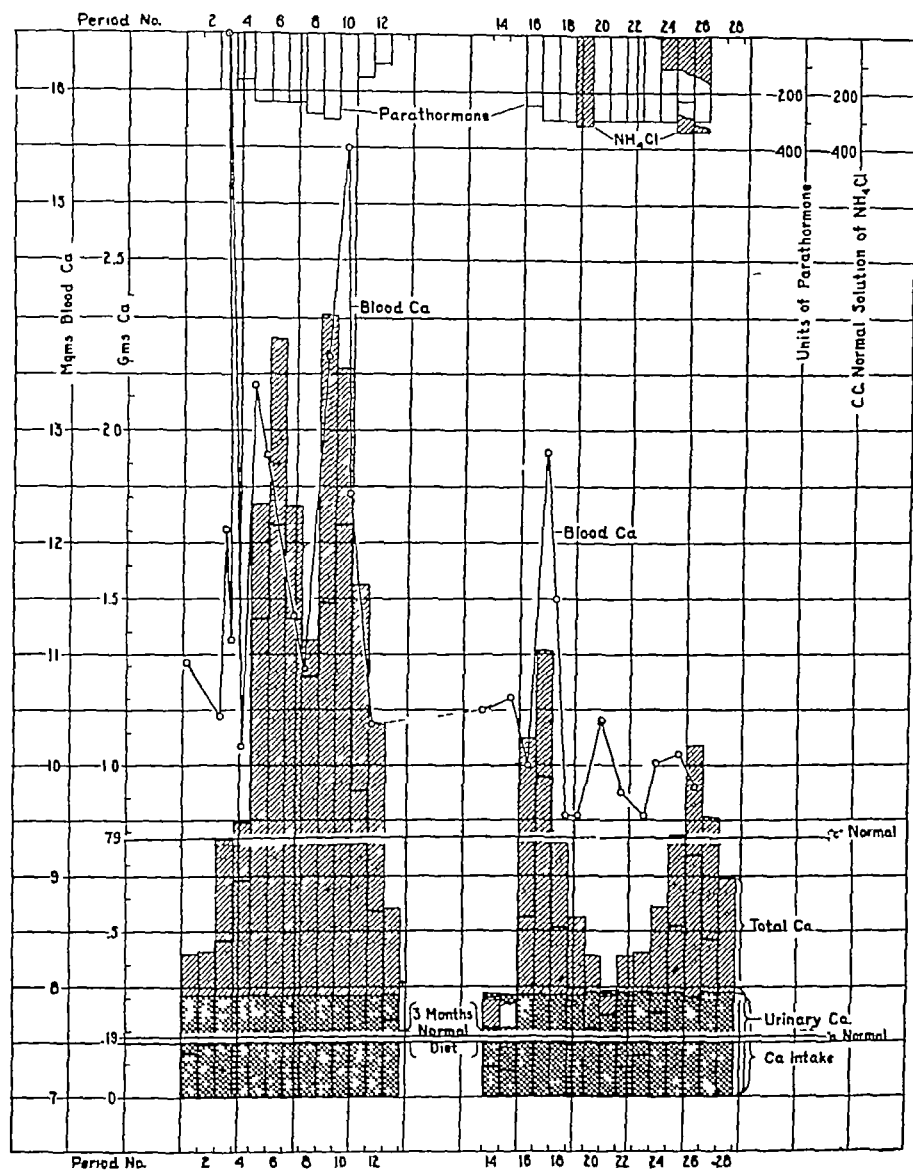
CHART IIa CALCIUM METABOLISM IN CASE II

medication and the calcium, phosphorus and nitrogen balances are recorded in charts IIa, IIb and IIc

The calcium data confirm the observations made on case I   The calcium rise (periods 3, 4, and 5) is more step like than in case I The negative calcium balance during the control periods (1, 2, 14,
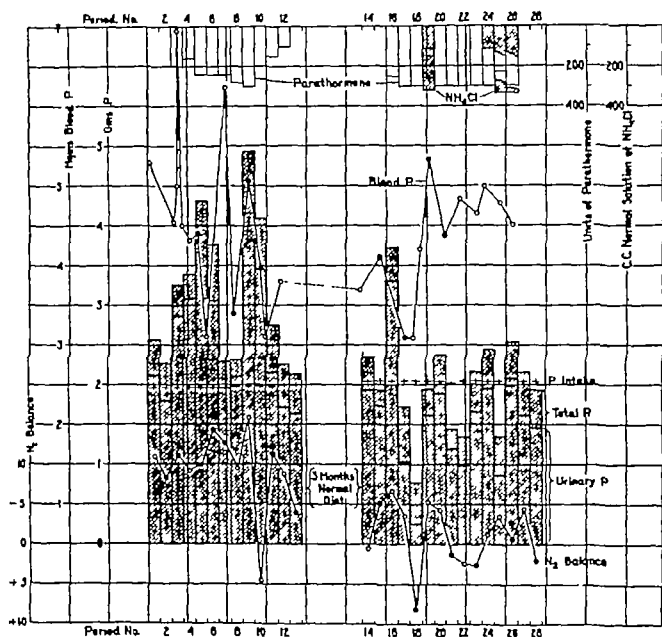
CHART IIb PHOSPHORUS METABOLISM IN CASE II

and 15) is very low, and actually becomes positive in periods 14 and 15 Whether this is due to the fact that the patient was growing or to the fact that he had otosclerosis has not been determined and does not affect the present discussion   The very high serum calcium in period 3 represents a determination taken in the afternoon and so is not com-
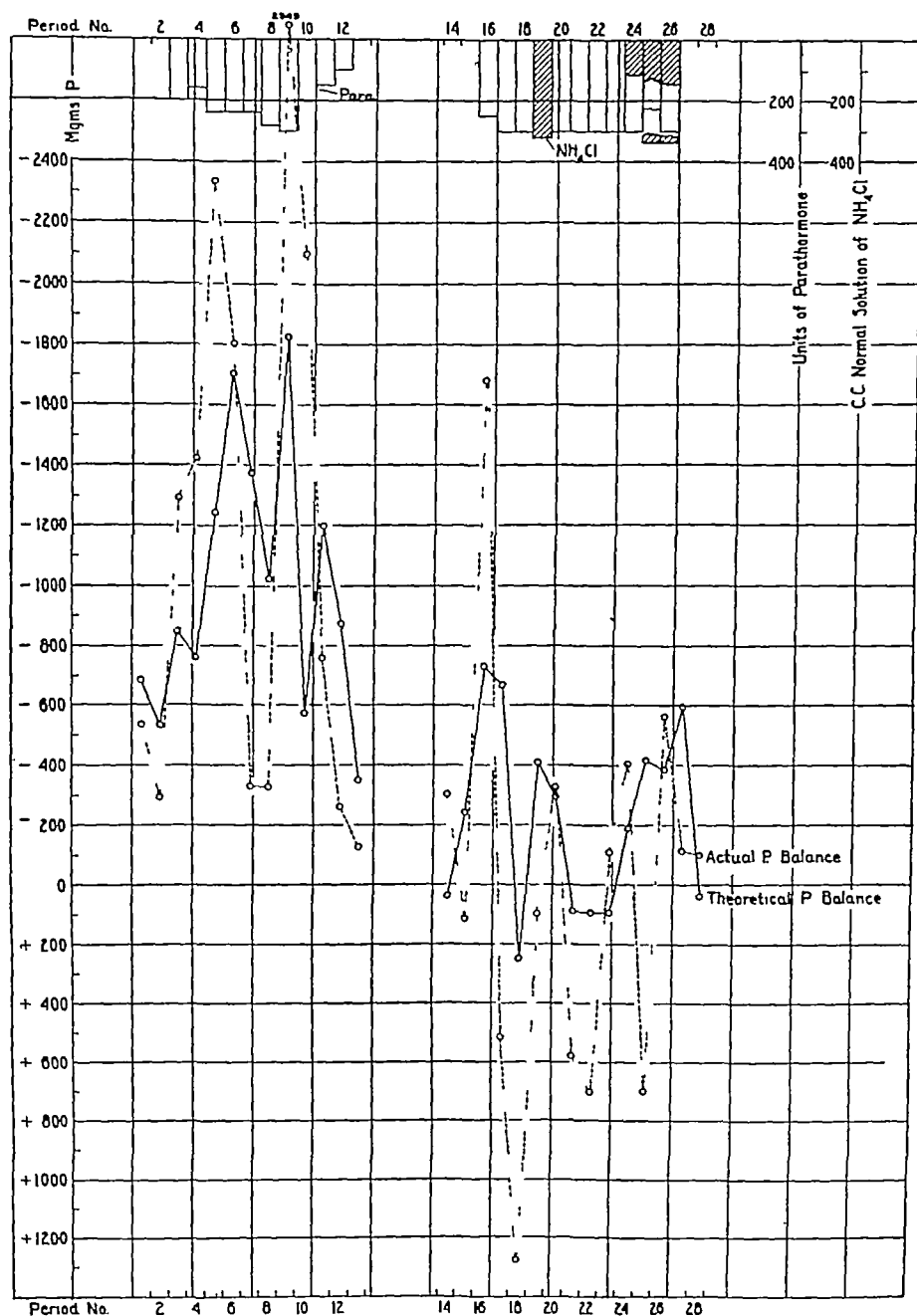
CHART IIc   "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN CASE II

parable with the other calcium determinations which represent morn-
ing values   This is important because the serum calcium level after
a dose of parathormone varies over a period of hours, so that in a
study like this the injections and the blood examinations should be
made at a fixed time each day   It should be noted that the calcium
response to parathormone was much greater in this than in the previ-
ous case.   In the second half of the experiment, the response to para-
thormone, after a satisfactory start in periods 16 and 17, completely
wears off in periods 18 to 23   This is apparently not due to a lack of
available calcium as there is a good response to ammonium chloride
in periods 24 to 26   The calcium response to ammonium chloride,
like the calcium response to parathormone, is almost entirely urinary

The phosphorus data (charts IIb and IIc) also corroborate the ob-
servations on case I   Parathormone affected the phosphorus excre-
tion more abruptly than it did the calcium   The initial rise in the
first periods of medication and the rapid return to normal on cessation
of the drug is more precipitous in the phosphorus figures   This is
brought out more clearly when comparing the actual phosphorus out-
put with the theoretical figure   In the first response to parathormone
the actual phosphorus excretions exceed the theoretical expectations,
and are less than the theoretical expectations when the parathormone
response disappears   Phosphorus excretions above the theoretical
appear to be associated with a falling blood phosphorus and to be
accompanied in the following period by a rising calcium excretion
During the second admission the same principles prevail, namely,
the actual phosphorus excretion exceeds the theoretical phosphorus
excretion during the first period of parathormone administration and
is accompanied by a falling serum phosphorus, then as the parathor-
mone becomes ineffective the actual phosphorus excretion falls below
the theoretical phosphorus excretion and the serum phosphorus rises
to an unusually high level   The effect of the ammonium chloride on
the phosphorus excretion is rather inconclusive   The tendency seems
to be for the urinary phosphorus to rise, which was to be expected
(4) (11) (see also cases III and VI below)

The additional observations to be noted from case II are

(xv) The effect of parathormone injection on the urinary calcium

excretion and the blood serum calcium may wear out completely This has been corroborated by our studies of tetany (5)

(xvi) This impotency of parathormone is apparently not due to an exhaustion of the available supply of calcium, because the calcium excretion can still be increased in a normal manner by ammonium chloride ingestion

(xvii) The first evidence of a cessation of the effectiveness of parathormone is a fall in the actual phosphorus excretion below the theoretical phosphorus excretion

(xviii) With ammonium chloride ingestion there is a rise in urinary calcium and phosphorus excretion

Case III   In case III, as in case II, we have further opportunity of studying what happens as parathormone injections lose their physiological effect   The subject was a colored boy of 14 who was suffering from an ossifying hematoma (myositis ossificans) of the right thigh, which had developed as a result of trauma three weeks before admission   In order to see whether parathormone could mobilize calcium from regions other than the bones, we gave the patient a low calcium diet with parathormone injections   No metabolism studies were made until the patient had been on this regime for 16 days, though there was obviously a fair response to parathormone as judged from the fact that the blood serum calcium rose to a maximum of 13 mgm The patient was then studied for 20 three-day periods while still on a low calcium diet   He was sent home for four months on a normal diet, and finally was studied again for 9 three-day periods on a low calcium diet   The medication and the calcium, phosphorus, and nitrogen data are given in charts IIIa, IIIb, and IIIc   During period 2 the patient had an attack of tonsillitis but his temperature remained elevated only 24 hours   He developed generalized urticaria during period 25, which cleared up promptly when parathormone was discontinued   All of these efforts to eliminate body calcium produced no change in the density of the ossifying hematoma as demonstrated by x-ray

An inspection of chart IIIa shows that in spite of increasing doses the effect of parathormone on the serum calcium and urinary calcium excretion was gradually lost   It is to be noted especially that the calcium excretion falls not only to what it would be without parathor-

mone but even to a much lower level, so that finally a positive balance is obtained in the latter half of the observation

The second part of this observation follows a rest period of four months   During this time he grew very markedly, and this may be
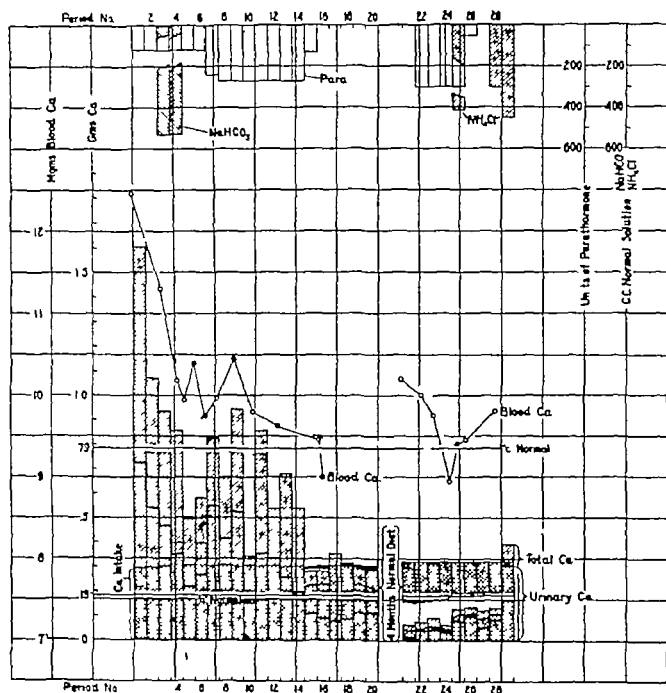
CHART IIIa. CALCIUM METABOLISM IN CASE III

the cause for his having the lowest calcium excretion we have observed in normal individuals on this test diet   In this second observation large doses of parathormone again fail to affect the calcium excretion, and the blood calcium even falls, although ammonium chloride still

proved effective   Thus the observation is corroborated that mobiliza-
tion of calcium can still be accomplished though parathormone proves
ineffective

The effect of parathormone on the phosphorus metabolism is
similar in kind to that seen in cases I and II   On his second admis-
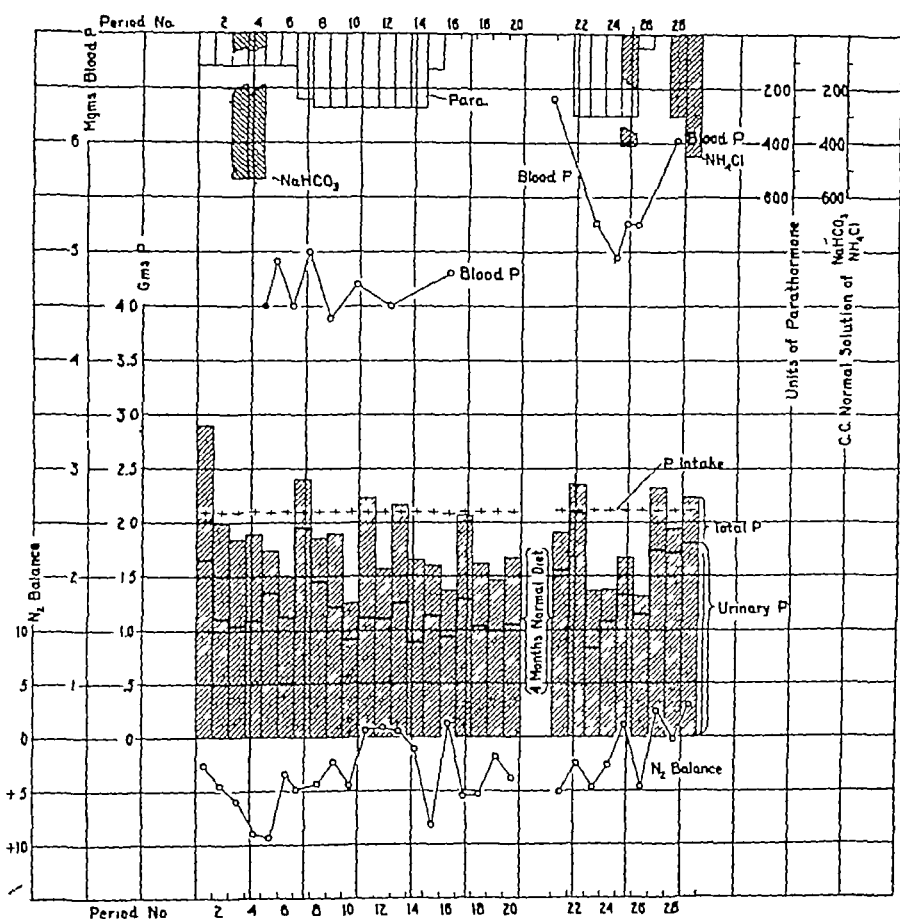
CHART IIIb PHOSPHORUS METABOLISM IN CASE III

sion, however, it is of interest to note the high initial serum phosphorus
level, which may be due to the season of the year (7) and to the fact
that he was growing (7)   As parathormone is administered (period
22), in spite of no effect on the calcium metabolism, there is some rise

in phosphorus excretion, an actual phosphorus excretion in excess of the theoretical phosphorus excretion (chart IIIc), and a corresponding fall of the blood phosphorus   Thus far, if we judge from the phosphorus data, the parathormone effect has been classical.   But the
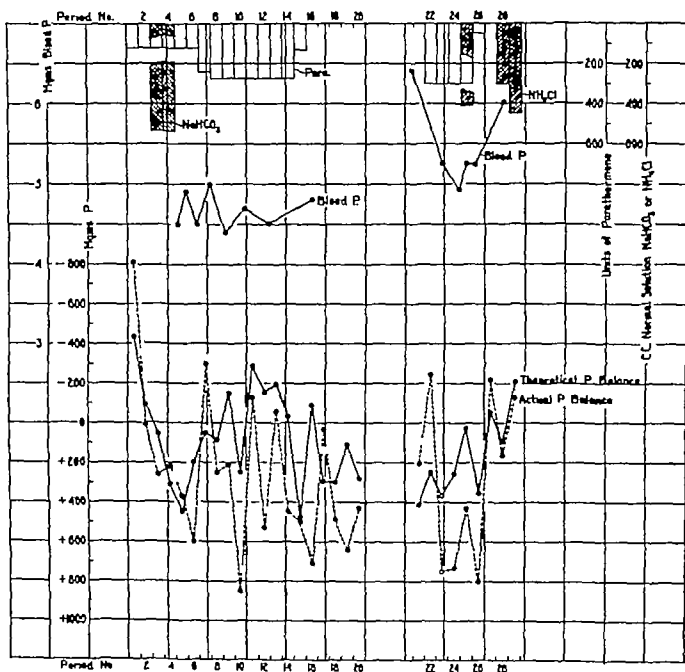
CHART IIIc. "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN CASE III

increase in the actual phosphorus excretion over the theoretical phosphorus excretion in period 22 is not followed by a rise in the calcium excretion   The increased phosphorus excretion lasts only for one period and then falls just as in period 17 of case II (chart IIb)   It is of interest that the data of Robinson, Huffman and Burt (25) on the

effect of parathyroid extract on normal calves show one case in which a classical phosphorus response was obtained without any concomitant calcium response As parathormone is discontinued and ammonium chloride is given, the serum phosphorus again rises to the pre-parathormone level (chart IIIb)

From case III we learn

(xix) In a person who has had parathormone and who has become "immune" to it, the negative calcium balance on a low calcium diet is much reduced or even becomes positive (see also case II)

(xx) The phosphorus response to parathormone administration in a person who fails to show any calcium response is shortlived, but qualitatively normal in this case

(xxi) Sodium bicarbonate administered to this individual did not effect or decrease the effectiveness of parathormone

(xxii) Parathormone administration has not been found helpful in decalcifying an ossifying hematoma   (See also case VIII)

Case IV   The subject of this observation was an Italian laborer of 55 who was suffering from chronic lead poisoning   The effect of parathormone on his lead excretion has been reported as case I by Hunter and Aub (8)   We have selected 18 three-day periods on a low calcium diet to report here in more detail   The medication, calcium balance, and blood plasma calcium data are given in chart IV   The nitrogen and phosphorus balances were not determined

It will be seen that the plasma calcium and the calcium excretion (especially fecal) fall off markedly during the 8 periods (24 days) following parathormone administration   There is then an elevation of the calcium excretion and of the low plasma calcium following ammonium chloride ingestion as shown in the last three periods   The increased calcium excretion appeared largely in the feces

This experiment confirms observation (ii), that after there has been a marked loss of body calcium produced by parathormone, the negative calcium balance on a low calcium diet is reduced   It also gives us these additional observations

(xxiii) During calcium starvation over long periods of time, especially if there has been an additional loss of calcium due to periods of parathormone administration, the blood calcium tends to fall (see also case III—periods 21-29 on chart IIIa)

(xxiv) A low blood calcium level due to calcium starvation is raised by ammonium chloride (see also case III)
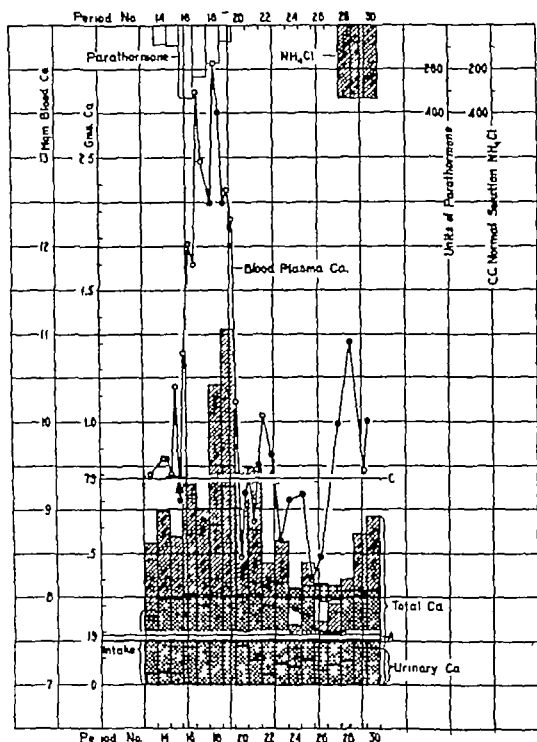
CHART IV CALCIUM METABOLISM IN CASE IV
The blood values in this case are on blood plasma

Case V   The subject of this investigation was a painter, aged 60, who was suffering from chronic lead poisoning with wrist drop   The results of the studies on lead and calcium excretions during periods

1–36 have been reported by Hunter and Aub (8) as their case IV
We have selected certain of these periods (4–14, 21–23, and 24–36)[4]
to report in greater detail and are reporting 19 additional periods on
the same patient (5'–24')   The data are shown in charts Va and Vb
It will be noted that periods 4–6 differ from all other periods thus far
discussed in that the low calcium in the diet is increased by the ad-
dition of calcium lactate.

The most striking part of these data (chart Va) is the extremely high
plasma calcium during periods 6, 7, and 8 produced by a dosage of
parathormone which was relatively small when compared with that
used in previous cases   Furthermore, at these high levels, the urinary
calcium excretion does not rise in proportion to the plasma calcium,
but remains approximately constant when the plasma calcium rises
above 14–15 mgm (cf 20 mgm in period 7)   The tendency for the
plasma calcium to fall below normal following cessation of parathor-
mone administration and to rise again with ammonium chloride
administration is well shown and is confirmatory of observation
(xvi) (v supra)   Turning to periods 5'–24' one notes that sodium
thiosulphate had no effect on either the calcium or the phosphorus
metabolism   This does not concern the present subject but is dis-
cussed in a paper dealing with the effect of sodium thiosulphate on
lead excretion (9) The response to parathormone in periods 10'
and 11' is classical   The phosphorus response in the urine is one full
period ahead of the calcium response and greater than can be explained
by the calcium-phosphorus ratio of bone   The slightly elevated blood
serum phosphorus value on the second day of period 11', correspond-
ing to the calcium value of 14 6, is in disagreement with the observa-
tion made in cases I–IV, where it was seen that the serum phosphorus
falls when the serum calcium rises   This type of reaction is apt to
occur when the calcium value has risen over 14 mgm and is associated
with a sudden diminution in phosphorus excretion   We believe this
is entirely a secondary phenomenon dependent on the high serum
calcium   It also accounts for certain sudden falls in the actual phos-
phorus excretion seen in most of the C charts which do not occur when
the calcium is kept at a lower level (cf case VII below)   The sodium

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[4] The patient left the hospital for one week between periods 23 and 24
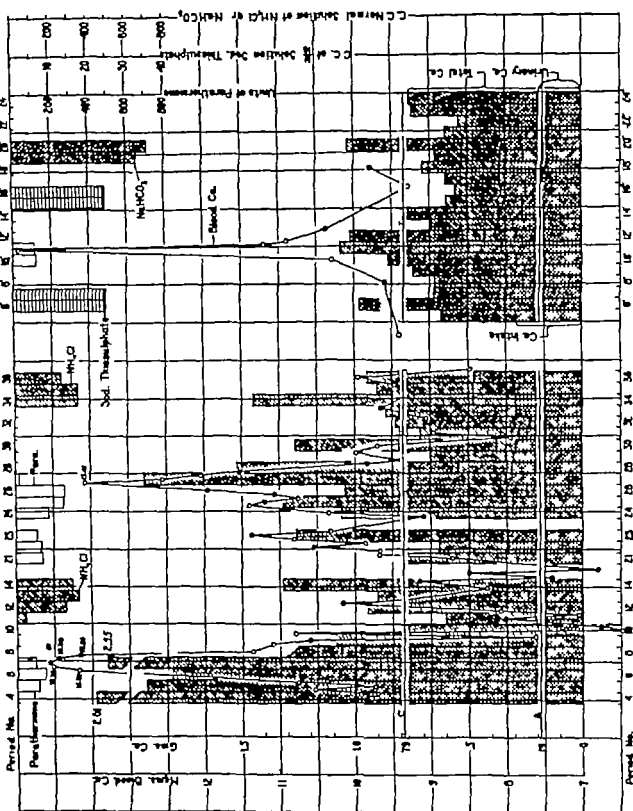
CHART Va CALCIUM METABOLISM IN CASE V

The blood values during the first admission are on blood plasma and during the second admission on blood serum

bicarbonate given in periods 19′ and 20′ increased both the urinary and fecal calcium and phosphorus, but especially the fecal phosphorus
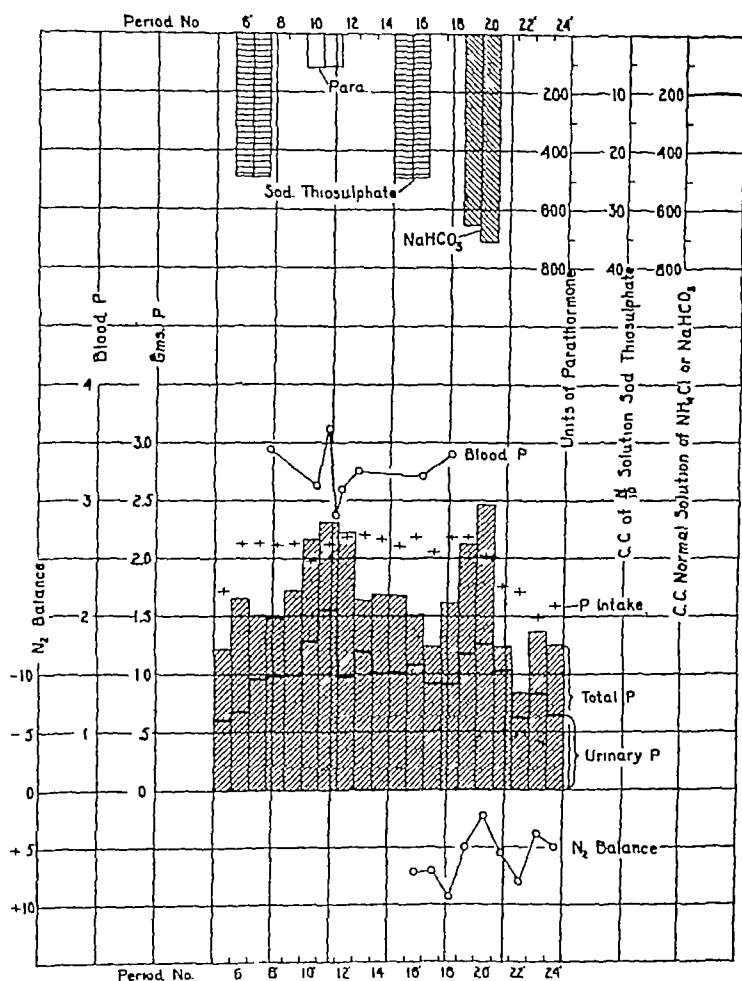
CHART Vb PHOSPHORUS METABOLISM DURING SECOND ADMISSION ON CASE V

(xxv) Parathormone has more effect on the blood calcium level of this patient than on that of any other in our series Its effect, therefore, may vary in different individuals

(xxvi) When parathormone is administered to a patient on a rela-

tively high calcium diet, the serum calcium rises more than if the patient were on a low calcium diet

(xxvii) When the serum calcium rises above 14–15 mgm , there is not a corresponding increase in the urinary calcium excretion

(xxviii) When the serum calcium rises above 14–15 mgm , the urinary phosphorus excretion falls and the serum phosphorus rises

(xxix) Sodium bicarbonate when administered alone causes a slight increase in the calcium and phosphorus excretions in the urine and especially in the feces (single observation—see also conclusion (xxi)) We do not differentiate here between excretion by and lack of absorption from the gastro-intestinal tract.

(xxx) Sodium thiosulphate given intravenously is without effect on the calcium and phosphorus excretions in this case.

Case VI   The subject for this experiment was a college man of 23 who had been afflicted with otosclerosis for ten years   He was studied for 14 periods of 3 days each, and he received parathormone injections for the long consecutive period of 36 days   The data of this experiment are tabulated in charts VIa, VIb and VIc

The phosphorus response to parathormone is unusually gradual but it still appears to be one period ahead of the calcium response   The excess of the actual phosphorus excretion over the theoretical phosphorus excretion is large (periods 5 and 6—chart VIc) and the response in the urinary calcium is correspondingly large   The tremendous increase in the urinary calcium excretion in periods 10 and 11, when ammonium chloride is given in conjunction with parathormone, is most striking   The total is probably beyond the sum of the increased excretions which would have been obtained had the drugs been administered separately   There was no marked change in the serum calcium as a result of the ammonium chloride   But what is perhaps the most important observation from a theoretical point of view is that the increases in the calcium and phosphorus excretions are coincident (chart VIc)   The excretion of the extra calcium does not extend into period 12   Thus when calcium phosphate is pulled from the bones by means of ammonium chloride, the calcium and the phosphorus are excreted in the same period   This is suggestive evidence that the excess phosphorus excretion above the theoretical, which is found at the beginning of parathormone administration, is due to an

CHART VIa   CALCIUM METABOLISM IN CASE VI

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CHART VIb PHOSPHORUS METABOLISM IN CASE VI

CHART VIIa CALCIUM METABOLISM IN CASE VII

CHART VIIb PHOSPHORUS METABOLISM IN CASE VII

CHART VIIc "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN
CASE VII

falls below the theoretical phosphorus excretion to about the same degree as it rises above this level at the beginning of parathormone administration

The new observation to record from this experiment is

(xxxv) During parathormone administration the phosphorus excretion tends to reach its highest level in the first periods

Case VIII   The previous experiments have been confined almost entirely to the study of calcium and phosphorus metabolism during parathormone administration and to a few isolated observations during the ingestion of acids and alkalies   In view of the conception of bone calcium as a reserve supply of fixed base, elaborated by Gamble (10) (11), it seemed wise to study at least on one patient, the total acid-base changes in the urine, in order to determine whether the increased mobilization of calcium phosphate with parathormone administration is associated with the formation of some acid radicle, as well as to investigate the effects of this drug on inorganic salts other than calcium and phosphorus

The patient was a laborer of 45 who, like case III, was receiving parathormone in the hope that it would decalcify a large ossified hematoma of three months' duration   No definite diminished density by x-ray could be demonstrated in the lesion following the parathormone therapy here described   This result is similar to that in case III   The patient ate exactly the same diet during the 42 consecutive days of the investigation   He was also on a constant fluid intake   The determinations included calcium, phosphorus, and total base analyses on the urine and feces, sulphate, chloride, and ammonia analyses on the urine, and total base, calcium, and phosphorus analyses on the diet   We also determined what we have called "the titratable acidity minus CO_2"   It was obtained by adding a known amount of $N/10$ hydrochloric acid to the urine, aerating until all the CO_2 was driven off, and then titrating back to a pH of 7 35   The value was obtained by subtracting the amount of acid used from the amount of alkali used in the titration   The details of the other methods employed have been discussed in a previous paper (2)   The data for experiment VIII are given in charts VIIIa, VIIIb, VIIIc, VIIId, VIIIe, and VIIIf

CHART VIIIa CALCIUM METABOLISM IN CASE VIII

Charts VIIIa, VIIIb, and VIIIc correspond to previous charts except that in charts VIIIb and c the actual phosphorus balance was obtained by using the determined phosphorus intake rather than the estimated phosphorus intake   The determined phosphorus intake
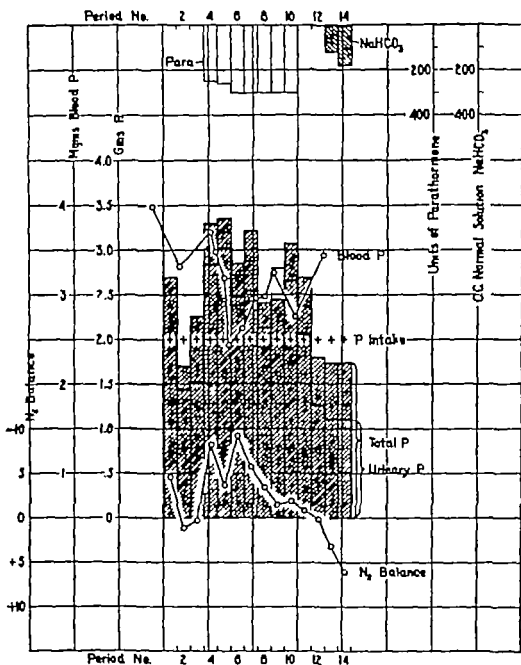
CHART VIIIb PHOSPHORUS METABOLISM IN CASE VIII

per three-day period was 300 mgm lower than the estimated phosphorus intake   This gives an indication of the possible error in the estimation of the phosphorus intake in the previous C-charts, and re-emphasizes the fact that the relative changes in the actual and theoretical phosphorus balances from period to period are more significant

than the absolute differences in any one period   Charts VIIIa,
VIIIb, and VIIIc offer further evidence in favor of previous observa-
tions but add no new observations   The sodium bicarbonate seems
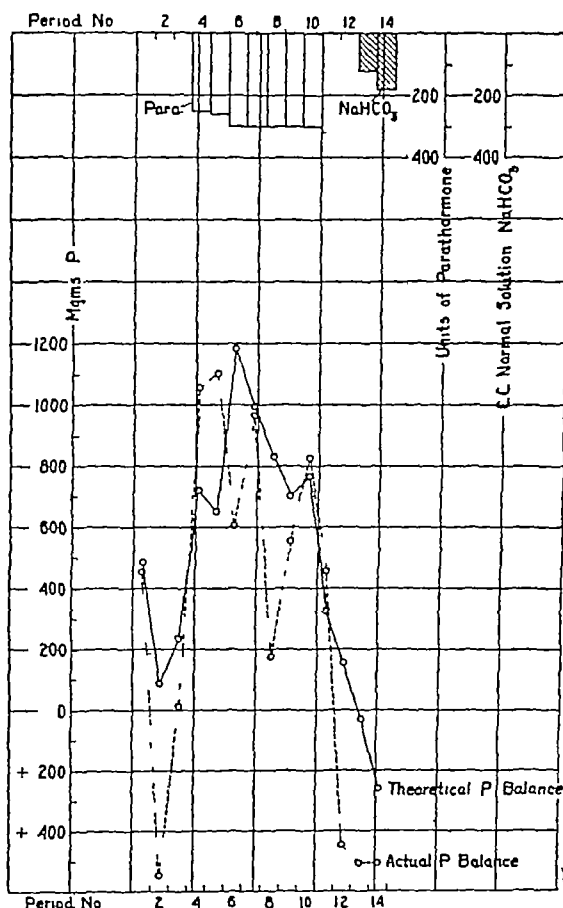
CHART VIIIc "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN
CASE VIII

to have had very little effect on the calcium and phosphorus metabo-
lisms (periods 13–17)

In chart VIIId the effect of parathormone therapy upon the total
base balance is recorded   The experiment checks very well in that the
total base ingested during the entire experiment is almost identical

with the total base excreted.   With parathormone one notes a rise in
the urinary excretion of total base, especially periods 3, 4, 8, 9, 10
In the first period following cessation of parathormone injections
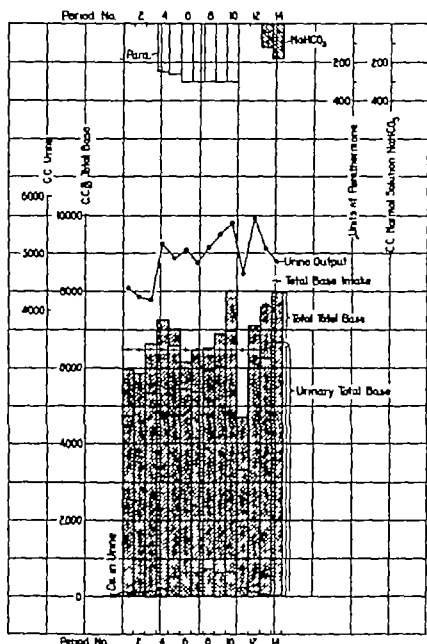
CHART VIIId   TOTAL BASE METABOLISM IN CASE VIII

All values for base are in cubic centimeter of N/10   This chart also shows the
urinary output on a scale chosen such that 1000 cc of urine is equivalent to 1500
cc of N/10 base (cf 1000 cc of body fluid is equivalent to about 1600 cc N/10
base)

there was an extreme drop in the total base excretion which suggests
that in this period there was a compensatory retention of total base
But before we can say that this represents an independent action of

parathormone on total base excretion, certain calculations are necessary   It seems fair to assume that the theoretical negative total base balance should equal the total base value of the calcium liberated from the bones plus the total base in the water held by destroyed muscle
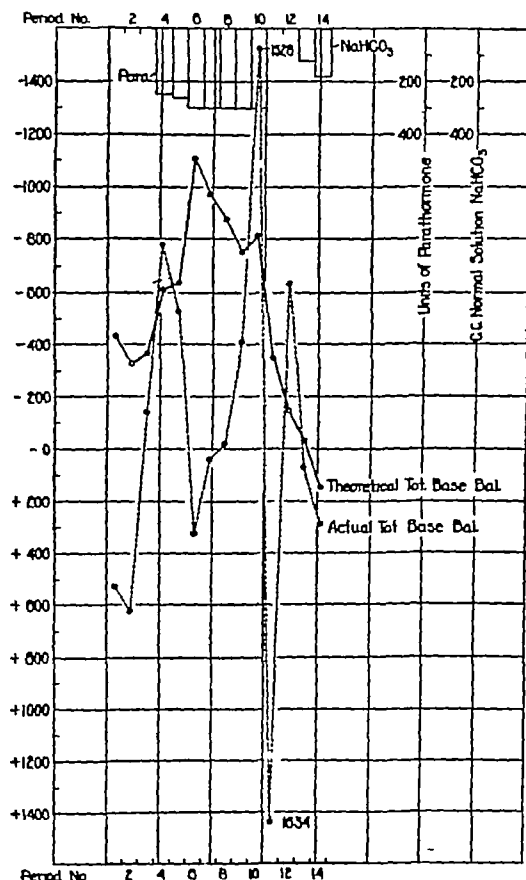
CHART VIIIe "THEORETICAL" AND ACTUAL TOTAL BASE BALANCE IN CASE VIII

(10)   Theoretically, any excess excretion of total base beyond this would be at the expense of the total base in the body fluids   We have calculated such a theoretical total base balance and in chart VIIIe this is plotted against the actual total base balance   Here, again, as in the corresponding phosphorus charts, it must be pointed out that
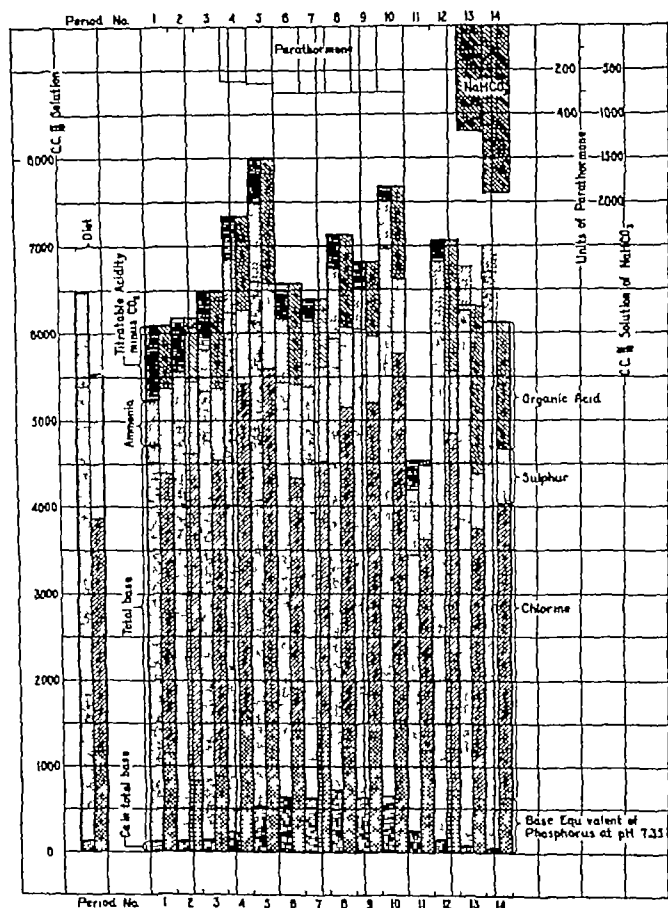
CHART VIIIf ACID BASE VALUES OF ANIONS AND CATIONS IN URINE IN CASE VIII

Acid-base properties of diet are represented at left. The chlorine and sulphur of the diet have been estimated from tables and so are only approximate The total base, calcium and phosphorus of the diet were actually determined

any small percentage error in determining the total base intake makes a large error in the actual base balance, so that the curve representing this may be incorrect by a uniform amount in all periods   In calculating the amount of muscle water liberated or stored from the nitrogen balance the following formula has been used

$$N \times 29\ 5 \times 0\ 76 = \text{muscle water (10)}$$

The first factor provides an estimate of the protoplasm destroyed and the second indicates the corresponding water content   The total base content of muscle water has been taken to be 180 cc N/10 per 100 cc (10)

On examination of chart VIIIe, one notes that the curve of the actual total base balance tends to be below the curve of the theoretical total base balance (448 cc N/10 by actual determination) due probably to an error in determining the total base intake   When charts VIIId and VIIIc are compared, it will be noted that the deviations of the actual from the theoretical excretions coincide fairly well   This leads one to believe that when phosphorus is excreted from the body fluids as a result of parathormone, there is also an excretion of total base held in the body fluids

Finally chart VIIIf has been made   Here the cations of the urine have been balanced against the anions in order to see whether any other anion or cation is affected by parathormone   The principles employed in balancing the electrolytes of the urine are those used by Gamble (10) (11), (12), except that the columns representing the cations and those representing the anions are both shorter by the CO_2 value of the urine than the corresponding ones of this author   The organic acid value is taken as the difference in the heights of the columns thus established   In periods 13 and 14 the values for the "titratable acidity minus CO_2" are negative due to the alkaline urine which resulted from sodium bicarbonate ingestion (12)   The values for the intakes are shown on the left of chart VIIIf   Calcium, total base, and phosphorus were actually determined in the intake   Sulphur and chlorine were estimated from tables   The chlorine value in the intake is obviously too low, but this is not surprising as the chlorine content of foods varies considerably

The deductions to be drawn from chart VIIIf are mostly negative

ones   The sulphur excretion rises in periods 4, 5, 6, and 7, but so does the negative nitrogen balance.   The chlorine varies considerably and tends to follow the total base   This is especially obvious in the fluctuation in periods 9, 10, 11, and 12   Greenwald (13) found a decreased excretion of chloride in the first two days following parathyroidectomy in dogs   The urinary ammonia rose slightly and the titratable acidity fell slightly when parathormone was administered. The organic acid values, which of course contain all the errors of the experiment, show no constant changes

The chief observation made in this case is a negative one, namely that

(xxxvi) The principle action of parathormone is confined to the calcium and phosphorus metabolisms

Other observations have not been fully proven but nevertheless are suggested   These are

(xxxvii) During parathormone administration, total base, water and chlorine tend to be excreted from the body fluids coincidentally with the excretion of phosphorus from the body fluids and tend to be retained in the body fluids during the periods of phosphorus retention

(xxxviii) The sulphur, ammonia and titratable acidity of the urine are little affected by parathormone

DISCUSSION

These observations show quite clearly the metabolic effects of parathormone on the organism.   It is an active preparation which has an unequal effect in different individuals   In several of our cases, reported here and in other papers, these effects soon wore off, and thereafter even large doses had apparently no obvious results   This is an important factor in its prolonged use for medication   The most obvious effect of parathormone, as previously reported by others, is in raising the blood calcium level and thereby increasing the urinary calcium excretion   At first it does this very effectively, but, while it is the only known method for raising appreciably the blood calcium, its effect on the calcium excretion is not as great as that found in thyrotoxicosis (14)   It is unfortunate that the effect of parathormone on both calcium and phosphorus metabolism is gradually lost   This

immunity to the drug has been found to remain at least a year in a normal individual and in a patient with tetany

There is evidence among these data that the primary effect of parathormone is on phosphorus excretion rather than on calcium Thus, when parathormone is administered, the first metabolic changes are a rise in phosphorus excretion and a fall in serum phosphorus, when parathormone is discontinued, the converse is true   At both times the changes in the rate of calcium excretion and in the height of the serum calcium level are more sluggish and lag behind about one period (3 days) (observation (v))   These time relationships are brought out by a study of the "theoretical" and "actual" phosphorus excretions (cf C-charts)   The actual phosphorus excretion is in excess of the calculated at the beginning of parathormone administration, and below, following cessation of parathormone   This implies that the extra phosphorus is drawn from the body fluids   It is also interesting that in cases II and III the wearing out of the parathormone effect was manifested first in the phosphorus metabolism   In the second admission of case III (period 21), although parathormone produced no effect on the calcium metabolism, there was a definite but poorly sustained effect on the serum phosphorus and on the phosphorus excretion   This strongly supports the hypothesis that the primary effect of parathormone is on phosphorus

The above discussion refers only to the primary effect of parathormone on phosphorus metabolism   It was emphasized first by Collip that when a certain critical level of serum calcium has been reached following parathormone injection, the blood phosphorus rises abruptly. This is probably due to an alteration in kidney function resulting from the high serum calcium, for the non-protein nitrogen in the blood also rises   Thus a late effect of parathormone on phosphorus metabolism is brought about, which is exactly opposite to the primary effect

There is evidence that the relatively greater excretion of phosphorus over calcium following administration of parathormone cannot be attributed to its more ready excretion through the kidney   The situation is not like the sulphur from protein metabolism which is excreted more rapidly than the nitrogen, for in case VI, when a large amount of $Ca_3(PO_4)_2$ was withdrawn from the bones by ammonium chloride, there was no lag in the excretion of the calcium behind that

of the phosphorus   Furthermore, when thyroid was given to normal controls (14), the increased excretion of calcium and phosphorus ran fairly parallel

There are several other observations which lend support to the possibility that the primary action of parathormone is on phosphorus Greenwald (13), who has always emphasized the phosphorus changes, has shown that there is a marked retention of phosphorus in the first two days after parathyroidectomy   The well known deleterious action of a meat diet on parathyroid tetany (15) (16) may be due to its high phosphorus content (17)   Collip (18) showed that following parathyroidectomy in the rabbit the serum phosphorus tended to rise very high without there being much change in the serum calcium, and that parathormone, if administered directly following operation, would prevent this rise   In conclusion he stated that, "these observations tend to emphasize the importance of phosphorus in relation to the pathogenesis of tetany "   Binger (19) decreased the serum calcium levels in dogs by intravenous injection of phosphates and produced tetany   Salvesen, Hastings and McIntosh (20) likewise produced tetany in dogs by feeding huge amounts of phosphates   This experiment is the exact antithesis to the report of Palmer and Eckles (21) wherein hypercalcemia, hypophosphatemia and osteomalacia in cows resulted from low phosphorus diets   These last two experiments are closely analogous to the situation in hypoparathyroidism and hyperparathyroidism respectively   In the first instance, instead of a decreased phosphorus output, there is an increased phosphorus intake, and in the second, instead of an increased phosphorus output there is a decreased phosphorus intake   The end results are similar Therefore, the fact that conditions similar to hypoparathyroidism and hyperparathyroidism can be brought about by influencing, through diets, the phosphorus balance, is strong evidence that it is primarily through changes in phosphorus metabolism that the states of hypo- and hyper-parathyroidism are reached

The observation in case VIII (observation (xxxvii)), that there is a tendency to an excretion of base, water, and chloride from the body fluids along with the loss of phosphorus and calcium, is supported by the recent use of parathormone as a diuretic in nephrosis   Its re-

ported good effects (22) (23) (24) in this disease may be due to an influence on the excretion of base from the tissue fluids

It is obvious that one effect of parathormone on the calcium-phosphorus metabolism is entirely different from that of acid formers like ammonium chloride or that of the thyroid hormone   Parathormone definitely elevates the blood level of calcium, while the others, which may increase the calcium excretion more than parathormone, do not significantly raise the blood calcium level, unless it is low as in tetany (5)

<div align="center">CONCLUSIONS</div>

The following may be stated as the main conclusions to be derived from this study of the effect of parathormone on patients maintained on a constant diet containing an inadequate amount of calcium

1 Parathormone administration *gradually* increases the urinary calcium excretion without affecting the fecal excretion   Following cessation of parathormone administration, the urinary calcium excretion *gradually* falls to a level below that found before the administration

2 The effect on calcium excretion of parathormone and ammonium chloride administered simultaneously is probably more than the sum of their individual effects

3 The calcium level in the blood is markedly but *gradually* elevated by parathormone injections   The extent of this elevation varies in different individuals, and is more marked when the patient is on a high calcium diet

4 Parathormone administration *abruptly* increases the urinary phosphorus excretion without affecting the fecal excretion   Following cessation of parathormone administration, the urinary phosphorus excretion *rapidly* falls to a level below that found before the administration   These changes are more rapid than those produced in the calcium metabolism, and greater than can be explained by a theoretical calculation of the phosphorus liberated with calcium and nitrogen

5 The phosphorus level in the blood is primarily lowered by parathormone   If, however, the serum calcium rises above a critical level of about 14 to 15 mgm , then the urinary phosphorus excretion falls and the blood phosphorus rises

6 These observations on the effect of parathormone suggest that an increased phosphorus excretion is the primary effect   The excreted phosphorus is partly derived from the body fluids

7 The effect of parathormone in some cases gradually wears off and the first evidence of this is found in a decreased phosphorus elimination   That this lack of response to parathormone is not due to an exhaustion of calcium reserves, is shown by the fact that ammonium chloride ingestion can still produce an increased elimination in such cases

8 Nitrogen excretion is not affected by parathormone

9 Parathormone has not helped to decalcify two ossifying hematomas

10 The effect of parathormone on other electrolytes is discussed, but no conclusions are formulated

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STUDIES ON THE PHYSIOLOGY OF THE PARATHYROID GLANDS

I. Calcium and Phosphorus Studies on a Case of Idiopathic Hypoparathyroidism

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INTRODUCTION

In order to determine, if possible, the exact train of events which lead to a rise in the serum calcium following the injection of a potent parathyroid extract, we undertook the present series of investigations. The subject of this study was an Italian boy on whom the diagnosis of idiopathic hypoparathyroidism was made. The criteria on which such a diagnosis can be based will be discussed below.

The injection of a potent parathyroid extract results in four well established changes and any theory as to the action of a parathyroid extract will have to take cognizance of these four cardinal points. They are

 a Rise in serum calcium (1)

 b Rise in urinary calcium excretion (2) (3) (4)

 c Fall in serum phosphorus (5)

 d Rise in urinary phosphorus excretion (2) (3)

Removal of the parathyroid glands results in the converse changes, i e ,

 a Fall in the serum calcium

 b Fall in the urinary calcium excretion

 c Rise in the serum phosphorus

 d Fall in urinary phosphorus excretion

In addition Albright, Bauer, Ropes, and Aub (3) emphasize the fact that on administration of parathormone, the increase in urinary phosphorus excretion tends to precede the increase in calcium excretion. They further point out that the increased phosphorus appearing in

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the urine must come partly from phosphorus dissolved in body fluids and not entirely from the calcium phosphate of the bones   Inasmuch as the calcium and phosphorus changes are probably not simultaneous, it was apparent that, by reducing the metabolism periods to eight hours instead of three days, as in the investigations above (3), we could follow this sequence of events more accurately (cf experiment 1 below)   Later we had occasion to reduce the period to 1 hour (cf experiment 2)   These shorter periods made it necessary for us to disregard the fecal excretions   This, however, is justifiable, as it has been shown that, following the injection of parathormone, there is a conspicuous alteration in the urinary excretion of calcium and phosphorus, but not in the fecal excretion of these elements (3)

REPORT OF CASE

We were fortunate in having the opportunity of studying the effect of parathormone on a patient suffering from idiopathic hypoparathyroidism   The clinical aspects of a case with this rather unique diagnosis are of interest

The patient, a school-boy of 14 of Italian descent, first entered the Medical Service of the Johns Hopkins Hospital on January 17, 1927 for relief of tetany (J H H No 9974).

The family history was non-contributory

The past history was uneventful except that he had had measles and chicken pox at 5 years of age

In 1918, at the age of 5, while under treatment for measles in the Harriet Lane Children's Clinic, it was noted that the patient had markedly hypertrophied tonsils   Tonsillectomy was performed in 1921   Shortly thereafter he began to be troubled at night with attacks of laryngismus   In August, 1922 he had his first attack of carpo-pedal spasm, precipitated by a gastro-intestinal upset   A second attack in January, 1923 was preceded by an upper respiratory infection   Examination at that time at the Harriet Lane Children's Clinic showed carpo-pedal spasm, a bilateral Chvostek sign, and an injected pharynx   The cathodal opening contraction was 1 0 milliampere   The serum calcium was 4 9 mgm per 100 cc and the serum phosphorus 10 4 mgm   The blood sodium chloride was 560 mgm per 100 cc

Ammonium chloride gave him symptomatic relief   However, the general course of the disease was downhill so that at the time of his first admission to the Medical Service he was having each day about fifteen attacks of carpopedal spasm   The individual attack was as a rule precipitated by some unusual exertion such as crossing the street or stealing a base in a ball-game   Momentary loss of consciousness was frequently associated with the attacks

Physical examination showed a normally developed Italian boy of healthy

appearance   The teeth were in poor repair   Both epitrochlear glands were palpable   Chvostek's and Trousseau's signs were positive

Other examinations showed a red blood cell count of 5,000,000, hemoglobin of 90 per cent (Sahli), white blood cell count 6600 with a normal differential count, a negative Wassermann reaction, a basal metabolism of minus six, and an entirely normal urine   The plasma calcium was 8 0 mgm per 100 cc. and the phosphorus 10 9 mgm per 100 cc.   The whole blood chlorides were 470 mgm. per 100 cc. and the CO_2 combining power 52 4 volumes per cent.

Following the administration of parathormone[1] there was complete relief   The serum calcium was raised to 16 2 mgm. and the serum phosphorus was lowered to 4 7 mgm   Symptoms returned, however, in one week after discontinuing parathormone

The present, the fourth admission to the Medical Service was on March 21, 1928 for relief for tetany   This time his symptoms had again, as frequently before, been aggravated by a pharyngitis   Examination this time showed in addition to the signs of tetany, a spleen which was just palpable   Ophthalmoscopic examination revealed a posterior, subcapsular, lenticular opacity and several peripheral, radially distributed, lenticular opacities in the right eye   In the left eye a few lenticular opacities were noted   The serum phosphorus was 10 8 mgm per 100 cc and the serum calcium 5.3 mgm per 100 cc.   X rays of the bones revealed nothing abnormal

The features of this case which make it possible to group it with those of hypo parathyroid tetany are

a Low serum calcium

b High serum phosphorus (in direct contradistinction to the infantile type of tetany where the serum phosphorus is low or normal or only slightly elevated)

c Cataracts which so commonly occur in post-operative hypoparathyroidism.

d Normal density of bones as shown by X ray (in contradistinction to instances of tetany associated with rickets and osteomalacia)

e Aggravation of tetany by exertion   We have found this to be characteristic in several cases of tetany of parathyroid origin

Thus we believe that, just as myxedema is, as a rule, an idiopathic hypothyroidism, so this case represents an idiopathic hypoparathyroidism

A report of a similar case is soon to be published from the Massachusetts General Hospital (6)   This, together with our case, presents exactly converse findings to those of another patient studied at the Massachusetts General Hospital, in whom a diagnosis of idiopathic *hyper*parathyroidism was made (7)

EXPERIMENT I

Experimental   This investigation lasted twenty seven days   An effort was made to have all factors as constant as possible   Conse-

TABLE 1
Experiment I

Day	Period	Fluid Intake cc	Fluid Output cc	Weight kgm	Calcium Intake mgm	Calcium Urine per 100 cc mgm	Calcium Total in urine mgm	Phosphorus Intake mgm	Phosphorus Urine per 100 cc mgm	Phosphorus Total in urine mgm	Serum Ca mgm per 100 cc	Serum P mgm per 100 cc	Units of para thor monet	Remarks
1	1	700	240		195	2 3	5 5	339	51 1	122				Tetany Chvostek
1	2	700	575		195	1 0	5 9	339	35 1	202				Tetany Chvostek
1	3	700	395		195	2 6	10 1	339	80 2	316				Tetany Chvostek
2	4	700	445	45 0	195	1 7	7 5	339	63 7	283				Tetany Chvostek
2	5	700	200		195	2 0	3 9	339	82 9	166	5 0	10 7		Tetany Chvostek
2	6	700	425		195	2 2	9 2	339	64 1	271				Tetany Chvostek
3	7	700	540		195	1 4	7 7	339	50 0	270				Tetany Chvostek
3	8	700	940		195	1 7	15 6	339	51 1	480				Tetany Chvostek
3	9	700	430		195	2 8	12 2	339	57 9	249				Tetany Chvostek
4	10	700	945		195	1 5	14 6	339	37 9	358	5 3	10 6		Tetany Chvostek
4	11	700	300		195	2 1	6 4	339	71 9	216	5 3	10 4		Tetany Chvostek
4	12	700	365		195	3 0	11 1	339	81 7	298	5 2	10 9		Tetany Chvostek
5	13	700	960	44 8	195	1 0	10 0	339	29 3	281				Tetany Chvostek
5	14	700	465		195	1 4	6 6	339	50 5	234				Tetany Chvostek
5	15	700	590		195	2 3	13 8	339	58 1	341				Tetany Chvostek
6	16	700	790	44 6	195	0 6	5 1	339	89 6	708	5 4	10 9	50	Tetany Chvostek
6	17	700	400		195	0 5	2 0	339	104 2	417	6 2	9 5		No tetany Chvostek negative
6	18	700	500		195	1 2	5 9	339	91 3	456	6 7	8 8		No tetany Chvostek negative

7	19	700	730	45 0	195	0 6	4 5	339	92 6	675	7 1	8 4	50	No tetany	Chvostek negative
	20	700	540		195	1 9	10 0	339	116 3	627	8 5	7 8		No tetany	Chvostek negative
	21	700	510		195	5 7	29 1	339	132 2	673		7 8		No tetany	Chvostek negative
8	22	700	720	44 6	195	3 4	24 8	339	84 6	608	9 4	7 0	50	No tetany	Chvostek negative
	23	700	460		195	13 5	63 3	339	122 5	564	9 8	6 6		No tetany	Chvostek negative
	24	700	635		195	14 8	94 3	339	77 9	495				No tetany	Chvostek negative
9	25	700	740	44 6	195	10 0	74 0	339	80 1	592	9 8	6 0	50	No tetany	Chvostek negative
	26	700	735		195	15 8	116 0	339	88 0	589	10 5	5 4		No tetany	Chvostek negative
	27	700	330		195	39 3	130 0	339	119 0	392	11 2	5 7		No tetany	Chvostek negative
10	28	700	610	43 8	195	8 5	51 8	339	40 2	245	10 1	5 7		No tetany	Chvostek negative
	29	700	415		195	6 8	28 2	339	43 5	181	9 3	5 9		No tetany	Chvostek negative
	30	700	360		195	10 0	35 9	339	37 4	135	8 8	6 5		No tetany	Chvostek negative
11	31	700	1,090	44 1	195	2 5	27 0	339	22 3	121	7 8	7 0		No tetany	Chvostek negative
	32	700	270		195	4 4	11 9	339	28 7	76	7 8	7 6		No tetany	Chvostek negative
	33	700	470		195	5 9	27 6	339	26 8	126				No tetany	Chvostek negative
12	34	700	820	43 5	195	3 1	25 5	339	19 2	157	7 0	8 6		No tetany	Chvostek negative
	35	700	140		195	4 3	6 1	339	53 1	74				No tetany	Chvostek negative
	36	700	425		195	11 1	48 5	339	57 3	243				No tetany	Chvostek negative
13	37	700	1,170	43 3	195	3 9	45 5	339	23 7	276	7 0	8 8		No tetany	Chvostek negative
	38	700	315		195	7 9	24 9	339	76 0	239				No tetany	Chvostek negative
	39	700	615		195	10 7	66 0	339	52 8	314				No tetany	Chvostek negative

* Serums for calcium and phosphorus determinations were taken at the beginnings of the periods in which they are tabulated.

† Prepared by Eli Lilly Company following Collip's specification

‡ Actual diets are Appended   Diet A was used in periods (1-42), diet B in periods (43-75), diet C in periods (79-81)

TABLE 1

Experiment I

Day	Period	Fluid Intake (cc)	Fluid Output (cc)	Weight (kgm)	Calcium Intake (mgm)	Calcium Urine per 100 cc (mgm)	Calcium Total in urine (mgm)	Phosphorus Intake (mgm)	Phosphorus Urine per 100 cc (mgm)	Phosphorus Total in urine (mgm)	Serum Ca (mgm per 100 cc)	Serum P (mgm per 100 cc)	Units of para thor mone	Remarks	
1	1	700	240		195	2.3	5.5	339	51.1	122				Tetany	Chvostek
	2	700	575		195	1.0	5.9	339	35.1	202				Tetany	Chvostek
	3	700	395		195	2.6	10.1	339	80.2	316				Tetany	Chvostek
2	4	700	445		195	1.7	7.5	339	63.7	283				Tetany	Chvostek
	5	700	200		195	2.0	3.9	339	82.9	166				Tetany	Chvostek
	6	700	425		195	2.2	9.2	339	64.1	271	5.0	10.7		Tetany	Chvostek
3	7	700	540		195	1.4	7.7	339	50.0	270				Tetany	Chvostek
	8	700	940		195	1.7	15.6	339	51.1	480				Tetany	Chvostek
	9	700	430		195	2.8	12.2	339	57.9	249				Tetany	Chvostek
4	10	700	945	45.0	195	1.5	14.6	339	37.9	358	5.3	10.6		Tetany	Chvostek
	11	700	300		195	2.1	6.4	339	71.9	216	5.3	10.4		Tetany	Chvostek
	12	700	365		195	3.0	11.1	339	81.7	298	5.2	10.9		Tetany	Chvostek
5	13	700	960	44.8	195	1.0	10.0	339	29.3	281				Tetany	Chvostek
	14	700	465		195	1.4	6.6	339	50.5	234				Tetany	Chvostek
	15	700	590		195	2.3	13.8	339	58.1	341				Tetany	Chvostek
6	16	700	790	44.6	195	0.6	5.1	339	89.6	708	5.4	10.9	50	Tetany	Chvostek
	17	700	400		195	0.5	2.0	339	104.2	417	6.2	9.5		No tetany	Chvostek negative
	18	700	500		195	1.2	5.9	339	91.3	456	6.7	8.8		No tetany	Chvostek negative

21	61	800	830	44 2	132	5 4	44 8	187	18 9	157	7 4	8 6	No tetany	Chvostek negative
	62	800	440		132	7 5	33 0	187	36 0	158			No tetany	Chvostek negative
	63	800	660		132	10 6	70 0	187	33 1	218			No tetany	Chvostek negative
22	64	800	630	44 1	132	6 4	40 3	1 149§	25 7	164	7 4	8 7	No tetany	Chvostek negative
	65	800	305		132	7 2	21 9	1 149	118 5	363			No tetany	Chvostek negative
	66	800	575		132	8 9	51 1	187	87 1	500			No tetany	Chvostek negative
23	67	800	825	44 0	132	3 8	31 0	668	51 8	478	7 1	9 6	No tetany	Chvostek negative
	68	800	625		132	3 2	20 0	668	73 4	460			No tetany	Chvostek negative
	69	800	625		132	6 6	41 2	668	73 6	462			No tetany	Chvostek negative
24	70	800	970	44 0	132	3 0	29 1	668	53 8	522	6 9	9 5	No tetany	Chvostek negative
	71	800	395		132	3 9	15 4	668	120 8	477			No tetany	Chvostek negative
	72	800	690		132	5 8	40 0	668	72 8	495			No tetany	Chvostek negative
25	73	800	930	44 0	132	2 8	26 0	668	54 6	508	6 9	10 5	No tetany	Chvostek negative
	74	800	520		132	3 3	17 2	668	91 0	490			No tetany	Chvostek negative
	75	800	590		132	5 8	34 2	668	92 8	547			-	Chvostek negative
26	76	800	380	44 0	619	4 4	16 7	1,255	128 2	487	7 0	10 3	No tetany	Chvostek negative
	77	800	500		619	4 6	23 0	1,255	105 0	525			No tetany	Chvostek negative
	78	800	470		619	14 6	18 7	1,255	116 7	548			No tetany	Chvostek negative
27	79	800	1,195	44 8	480	4 8	57 4	939	45 5	544	7 7	9 0	No tetany	Chvostek negative
	80	800	905		480	3 8	34 4	939	75 8	686			No tetany	Chvostek negative
	81	800	510		480	9 4	47 9	939	92 6	472	7 9	8 6	No tetany	Chvostek negative

§ Increased phosphorus obtained by adding NaH₂PO₄ to diet.

TABLE 1—Continued

Day	Period	Fluid Intake cc	Fluid Output cc	Weight kgm	Calcium Intake† mgm	Calcium Urine per 100 cc mgm	Calcium Total in urine mgm	Phosphorus Intake† mgm	Phosphorus Urine per 100 cc mgm	Phosphorus Total in urine mgm	Serum* Ca mgm per 100 cc	Serum* P mgm per 100 cc	Units of para thor mone†	Remarks	
14	40	700	760	44 0	195	4 4	33 4	339	33 8	257	7 1	8 7		No tetany	Chvostek negative
	41	700	420		195	5 8	25 0	339	62 1	261				No tetany	Chvostek negative
	42	700	615		195	10 9	67 0	339	59 0	362				No tetany	Chvostek negative
15	43	800	885	43 9	132	4 9	43 5	187	33 2	295	7 7	8 7		No tetany	Chvostek negative
	44	800	555		132	5 7	31 6	187	40 0	222				No tetany	Chvostek negative
	45	800	605		132	10 0	60 0	187	45 9	278				No tetany	Chvostek negative
16	46	800	730	43 7	132	4 0	29 0	187	27 2	198	7 3	8 8		No tetany	Chvostek negative
	47	800	610		132	4 2	25 6	187	38 9	237				No tetany	Chvostek negative
	48	800	700		132	9 8	68 6	187	30 8	216				No tetany	Chvostek negative
17	49	800	705	43 3	132	4 8	33 8	187	20 9	147				No tetany	Chvostek negative
	50	800	630		132	5 4	34 0	187	27 4	173				No tetany	Chvostek negative
	51	800	480		132	12 4	59 5	187	43 8	211				No tetany	Chvostek negative
18	52	800	830	43 7	132	5 2	43 2	187	22 0	182	7 7	8 6		No tetany	Chvostek negative
	53	800	560		132	5 5	30 8	187	31 8	178				No tetany	Chvostek negative
	54	800	595		132	11 9	70 8	187	37 7	222				No tetany	Chvostek negative
19	55	800	855		132	5 0	42 7	187	19 0	164				No tetany	Chvostek negative
	56	800	425		132	6 1	25 9	187	37 8	161				No tetany	Chvostek negative
	57	800	455		132	12 9	58 7	187	44 2	201				No tetany	Chvostek negative
20	58	800	905	44 0	132	4 2	37 9	187	18 2	165	7 5	8 8		No tetany	Chvostek negative
	59	800	455		132	5 6	25 5	187	34 8	158				No tetany	Chvostek negative
	60	800	630		132	10 8	68 0	187	33 0	211				No tetany	Chvostek negative

21	61	800	830	44 2	132	5 4	44 8	187	18 9	157	7 4	8 6	No tetany	Chvostek negative
	62	800	440		132	7 5	33 0	187	36 0	158			No tetany	Chvostek negative
	63	800	660		132	10 6	70 0	187	33 1	218			No tetany	Chvostek negative
22	64	800	630	44 1	132	6 4	40 3	1 199§	25 7	164	7 4	8 7	No tetany	Chvostek negative
	65	800	305		132	7 2	21 9	1 199	118 5	363			No tetany	Chvostek negative
	66	800	575		132	8 9	51 1	187	87 1	500			No tetany	Chvostek negative
23	67	800	825	44 0	132	3 8	31 0	668	51 8	478	7 1	9 6	No tetany	Chvostek negative
	68	800	625		132	3 2	20 0	668	73 4	460			No tetany	Chvostek negative
	69	800	625		132	6 6	41 2	668	73 6	462			No tetany	Chvostek negative
24	70	800	970	44 0	132	3 0	29 1	668	53 8	522	6 9	9 5	No tetany	Chvostek negative
	71	800	395		132	3 9	15 4	668	120 8	477			No tetany	Chvostek negative
	72	800	690		132	5 8	40 0	668	72 8	495			No tetany	Chvostek negative
25	73	800	930	44 0	132	2 8	26 0	668	54 6	508	6 9	10 5	No tetany	Chvostek negative
	74	800	520		132	3 3	17 2	668	91 0	490			No tetany	Chvostek negative
	75	800	590		112	5 8	34 2	668	92 8	547			No tetany	Chvostek negative
26	76	800	380	44 0	619	4 4	16 7	1,255	128 2	487	7 0	10 3	No tetany	Chvostek negative
	77	800	500		619	4 6	23 0	1,255	105 0	525			No tetany	Chvostek negative
	78	800	470		619	14 6	68 7	1,255	116 7	548			No tetany	Chvostek negative
27	79	800	1,195	44 8	480	4 8	57 4	939	45 5	544	7 7	9 0	No tetany	Chvostek negative
	80	800	905		480	3 8	34 4	939	75 8	686		8 6	No tetany	Chvostek negative
	81	800	510		480	9 4	47 9	939	92 6	472	7 9		No tetany	Chvostek negative

§ Increased phosphorus obtained by adding NaH₂PO₄ to diet.

quently the patient was kept in bed and in doors   Each day was divided into three eight-hour metabolism periods   The periods began at 8 a m , 4 p m , and 12 midnight   The patient was given the same meal three times a day at the beginning of each period   Water was likewise administered in equal amounts and at the same relative time in each period   The urine was collected for each period and analyzed for calcium[2] and for phosphorus (9)   Vena punctures when done were performed at the beginning of the periods   Fifty units of parathormone were administered at the beginning of periods 16, 19, 22 and 25 (cf chart 1)   The diet was altered at period 43 and several times thereafter as can be noted from table 1   The data for this experiment are given in table 1 and chart 1

Results   One notes in control periods 1 to 15 before the administration of parathormone a constantly high serum phosphorus, a correspondingly low serum calcium, and extremely low calcium excretion in the urine, and a phosphorus excretion in the urine comparable with that observed in normal persons on a similar phosphorus intake   This normal urinary phosphorus excretion is not at variance with the four cardinal points mentioned above or with the lowered phosphorus excretion following parathyroidectomy, demonstrated by Greenwald (10)   In our case the patient was in equilibrium at a constant level of hypoparathyroidism and was not shifting from a normal to a hypoparathyroid state, as in the post-operative cases   On administration of parathormone the urinary phosphorus excretion rose to its maximum, even exceeding the intake, in the first period (period 16), maintained this high level throughout the period of parathormone administration, and then fell abruptly following cessation of parathormone administration   The serum phosphorus fell coincidently with the increased phosphorus excretion and rose again when the phosphorus excretion fell off   The serum calcium rose as the serum phosphorus fell and vice versa with almost no tendency for one to lag behind the other   The calcium excretion failed to rise during the five periods following parathormone administration but then rose very abruptly

The calcium excretion fell off following cessation of parathormone

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[2] Fiske method described by Blackfan and Hamilton (8)

administration   It should also be noted that the calcium excretion in the urine actually fell slightly during the first five periods after the beginning of parathormone administration   Following cessation of
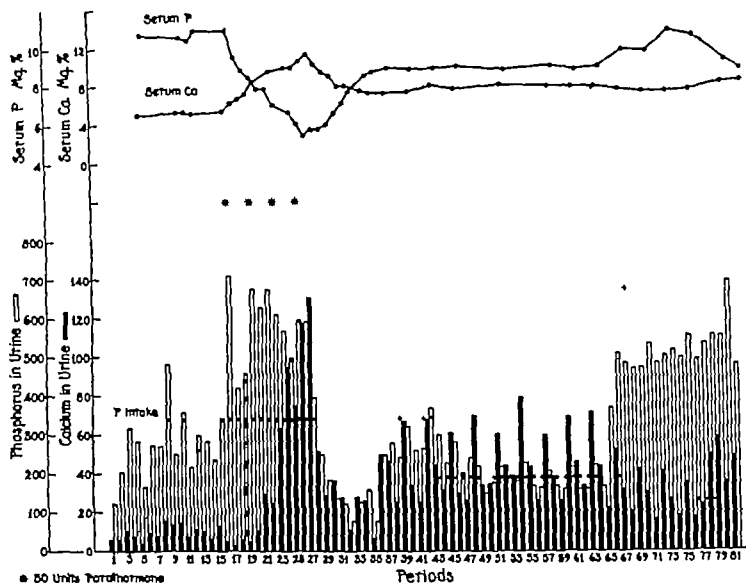
CHART 1 GRAPHIC REPRESENTATION OF DATA IN EXPERIMENT 1

The scale for the serum phosphorus is twice that for the serum calcium   This is so chosen because the normal value for serum calcium is about twice that for serum phosphorus, and because, consequently, a change of 2 mgm of calcium represents the same percentage deviation from normal as a change of one mgm of phosphorus   For convenience in charting, the scale for the urinary calcium excretion is five times that for the urinary phosphorus excretion   Since the ratio of calcium to phosphorus in bone is 2.23 a calcium scale one half that of the phosphorus would have been more logical, although not practical

parathormone administration the phosphorus excretion fell below the preparathormone level of excretion (cf periods 29 to 35)   This is analogous to the decreased phosphorus excretion following parathy-

roidectomy (10)   It may be significant that of these four variables, the phosphorus excretion alone seemed to depend quantitatively on the amount of parathormone given   The others seemed to depend on how long the parathormone was given and perhaps on how long this high level of phosphorus excretion was maintained

In periods 43 to 63 the phosphorus intake was decreased to a minimum in order to determine whether the serum phosphorus could thus be diminished   The result was a decreased phosphorus excretion with practically no change in the serum phosphorus or in the serum calcium   Likewise in periods 64 to 75, when the phosphorus intake was greatly increased by the addition of primary sodium phosphate to the diet, the urinary phosphorus excretion was greatly elevated while there was only a slight tendency for the serum phosphorus to rise and for the serum calcium to fall   Periods 76 to 81 will not be discussed as the changes in the diet were too radical

Comment   The observations of experiment I suggest certain conclusions, the interpretation of which may be as follows

1   A state of hypoparathyroidism is associated with a fairly normal urinary phosphorus excretion, a very high serum phosphorus, a low-serum calcium, and a very low urinary calcium excretion

2   Administration of parathormone to an individual in a state of hypoparathyroidism modifies all four of these factors, viz

a   The urinary phosphorus excretion immediately rises to its maximum (at least within eight hours)

b   Coincidently the serum phosphorus falls

c   The serum calcium rises

d   The urinary calcium excretion at first diminishes, and then when the serum calcium is about 8 5, suddenly increases

3   Cessation of administration of parathormone to an individual who had been brought from a hypoparathyroid state to the normal, further modifies these factors, as follows

a   The phosphorus excretion at once falls even below the preparathormone level

b   The serum phosphorus rises

c   The serum calcium falls

d   The calcium excretion falls, but less precipitously than the phosphorus excretion

4 Whereas the urinary calcium excretion ordinarily varies with the height of the serum calcium (3), there seems to be a threshold-level at about 8 5 mgm of calcium per 100 cc, below which, the calcium excretion in the urine becomes more or less constant and is independent of the serum calcium value

TABLE 2
Experiment II

Time	Fluid intake	Urine	Calcium in urine	Phosphorus in urine	Chlorine in urine (as NaCl)	Serum		Units of parathormone	Remarks
						Calcium	Phosphorus		
a.m.	*cc*	*cc*	*mgm*	*mgm*	*grams*	*mgm per 100 cc*	*mgm per 100 cc*		
7–8	250	85	2 8	7 2	0 59				Serum CO₂ 51 3 volumes per cent Serum NaCl 597 mgm. per 100 cc. Refractive index 1.3530
8–9	250	185	1 4	6 2	0 50	7 3			
9–10	250	230	1 7	6 4	0 69				
10–11	250	255	1 6	6 9	0 48	7 5	10 3		
11–12	250	510	3 3	39 5	1 07			75	
p.m.									
12–1	250	270	0 9	72 0	0 32	7 3	10 2		
1–2	250	220	1 0	62 7	0 20	7 5			
2–3	250	230	1 0	58 0	0 21	7 7	8 8		
3–4	250	275	0 8	73 7	0 28	8 1	9 3		
4–5	250	85	0 5	72 9	0 32	8 7	9 2		
5–6	250	240	1 1	33 1	0 22	8 7			Serum CO₂ 52.2 volumes per cent. Serum NaCl 591 mgm. per 100 cc. Refractive index of serum 1.3532
6–7						8 0	9 3		

* Blood for Ca and P taken at beginning of period tabulated

5 The high serum phosphorus, present in hypoparathyroidism, does not appear to be altered materially in a week's time either by a very low phosphorus intake or by a very high one

EXPERIMENT II

The impression has been prevalent that parathormone is a drug that acts slowly   Collip (1) points out that when parathormone is in-

jected into dogs the peak of the serum calcium curve is reached in twelve to twenty-four hours In experiment I, we have shown that the maximum effect on urinary phosphorus excretion was reached
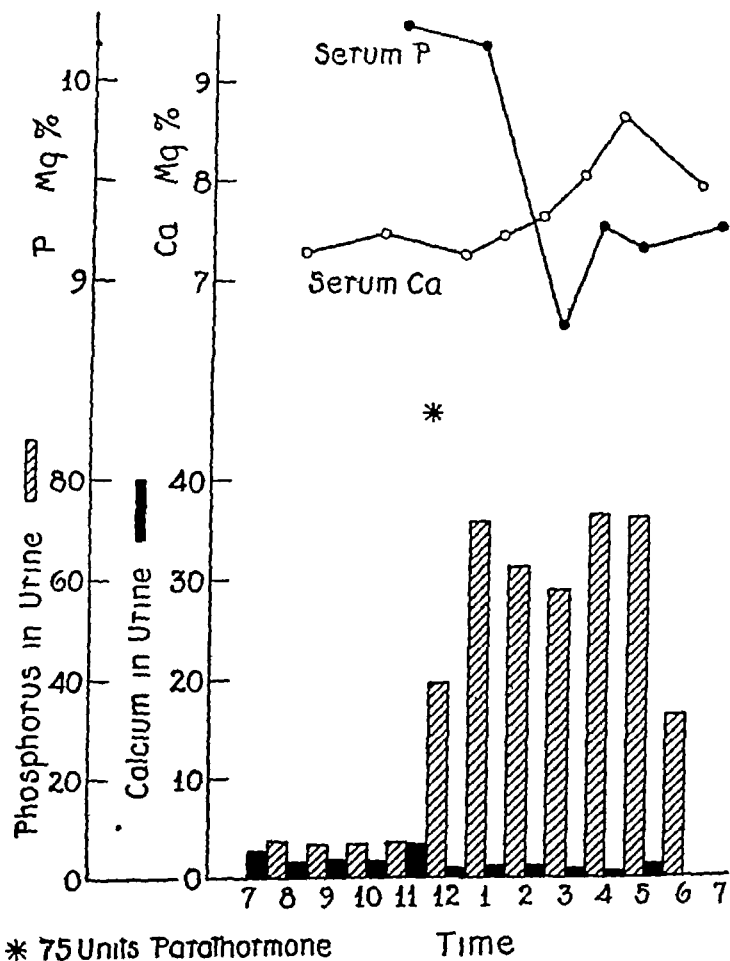
CHART 2 GRAPHIC REPRESENTATION OF CALCIUM AND PHOSPHORUS
DATA IN EXPERIMENT 2

The scale for the serum phosphorus is twice that for the serum calcium The scale for the phosphorus excretion in the urine is one-half that of the calcium excretion

somewhere during the first eight hours The second experiment was planned to determine whether this increased phosphorus excretion might occur even earlier than eight hours

Experimental   Following a period of 16 days on a low phosphorus diet (0 345 gram per day), the patient fasted and remained in bed for the day of the experiment   He received 250 cc of water at 7 a m and the same amount every hour thereafter until 6 p m when the investigation ceased   The urine was collected every hour and analyzed for calcium, phosphorus and chlorine   At 11 a m 75 units of parathormone were administered   The results can be seen in table 2 and chart 2

Results   It will be noted that the excretion of urinary phosphorus increased about 500 per cent in the first hour after parathormone administration and reached its peak by the second hour   In this experiment the serum phosphorus seems to have fallen more quickly than the serum calcium rose   Both of these values had altered within three hours of the injection   The calcium excretion was slightly decreased following the injection (cf experiment I)   The increased excretion of water and sodium chloride in the first hour after injection are interesting but will not be discussed here

Comment   6 The action of parathormone on the excretion of urinary phosphorus is maximal within the first hour after injection   It follows from this that future studies must concentrate on the changes occurring in the first hour after injection if one is to learn the fundamental mechanism,—not the remote end results

EXPERIMENT III

In the latter part of experiment I it was noted that changes in the amount of phosphorus in the diet had little effect on the serum phosphorus   It was desired to extend this investigation over longer periods of time because of its important theoretical significance

Experimental   This experiment started immediately after experiment I and lasted forty days   The time was divided into 10 four day metabolism periods   During the first six periods the patient was on a high phosphorus diet (1 756 gram per day) and during the remaining four on a low phosphorus diet (0 345 gram per day)   Except for the change from the high to the low phosphorus diet, the patient received the same food each day   He likewise received the same amount of water each day   He was not kept in bed   The urine for each period was examined for calcium, phosphorus and nitrogen, and the feces

TABLE 3

Experiment III

Period	Weight	Fluid intake	Urine volume — Day	Urine volume — Night	Urine volume — Total	Calcium Intake*	Ca Total day urine	Ca Total night urine	Ca Total urine	Ca Feces	Ca Total excretion	Ca Balance	Ca P equivalent of Ca balance	Phosphorus Intake*	P Total day urine	P Total night urine	P Total urine	P Feces	P Total excretion	P Balance	Nitrogen Intake*	N Total urine	N Feces	N Total excretion	N Balance	N P equivalent of N balance	Serum Calcium	Serum Phosphorus
	kg	cc	cc	cc	cc	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	mg per 100 cc	mg per 100 cc
1	44 1	5 920	1 060	3 950	5 010	3 976			0 581	4 080	4 661	−0 685	−0 307	7 024			5 090	3 39	8 480	−1 456	7 657	6 481	0 066	6 547	+11 1	+0 64	7 7	7 3
2	44 5	6 040	910	3 950	4 860	3 976	0 059	0 229	0 288	2 53	2 818	+1 058	+0 474	7 024	1 010	2 960	3 970	3 69	7 660	−0 636	6 658	6 592	0 066	6 658	0 0	0 00	6 5	8 7
3	44	6 040	1 020	4 125	5 145	3 976	0 041	0 177	0 218	6 16	6 378	−2 402	−1 077	7 024	1 480	3 120	4 600	3 69	8 290	−1 266	7 450	6 504	0 066	6 570	+8 8	+0 51	6 2	8 2
4	45 1	6 040	1 120	3 870	4 990	3 976	0 055	0 158	0 213	3 56	3 773	+0 203	+0 091	7 024	1 506	3 440	4 946	2 60	7 546	−0 522	6 874	6 568	0 066	6 634	+2 4	+0 14	6 0	8 3
5	45 2	6 040	1 440	3 690	5 130	3 976	0 048	0 129	0 177	4 08	4 257	−0 281	−0 126	7 024	2 190	2 790	4 980	2 70	7 680	−0 656	8 206	6 420	0 066	6 486	+17 2	+0 99	6 0	8 5
6	45 4	6 040	1 150	3 650	4 800	3 976	0 024	0 113	0 137	2 32	2 457	+1 519	+0 681	7 024	1 456	3 175	4 631	2 70	7 331	−0 307	7 279	6 523	0 066	6 589	+6 9	+0 40	6 0	9 0
7	45 5	5 380	1 800	3 050	4 850	5 402	0 027	0 088	0 115	4 00	4 115	+1 287	+0 577	1 380	0 452	0 924	1 376	1 44	2 816	−1 436	6 022	6 256	0 026	6 282	−2 6	−0 15	6 3	10 2
8	45 0	6 380	2 425	3 040	5 465	5 402	0 036	0 115	0 151	2 39	2 541	+2 861	+1 283	1 380	0 235	0 453	0 688	0 730	1 418	−0 038	6 526	6 200	0 026	6 226	+3 0	+0 17	6 5	10 6
9	45 0	6 380	2 625	2 920	5 545	5 402	0 039	0 117	0 156	5 24	5 396	+0 006	+0 002	1 380	0 160	0 233	0 393	0 730	1 123	+0 257	6 184	6 238	0 026	6 264	−0 8	−0 05	6 7	9 8
10	44 8	6 380	2 670	3 235	5 905	5 402	0 040	0 136	0 176	4 07	4 246	+1 156	+0 518	1 380	0 176	0 265	0 441	0 910	1 351	+0 029	6 580	6 194	0 026	6 220	+3 6	+0 21	7 7	9 7

* Actual diets appended   Diet D used in periods 1–6   diet E in periods 7–10   Ca and P values obtained by analysis of diets

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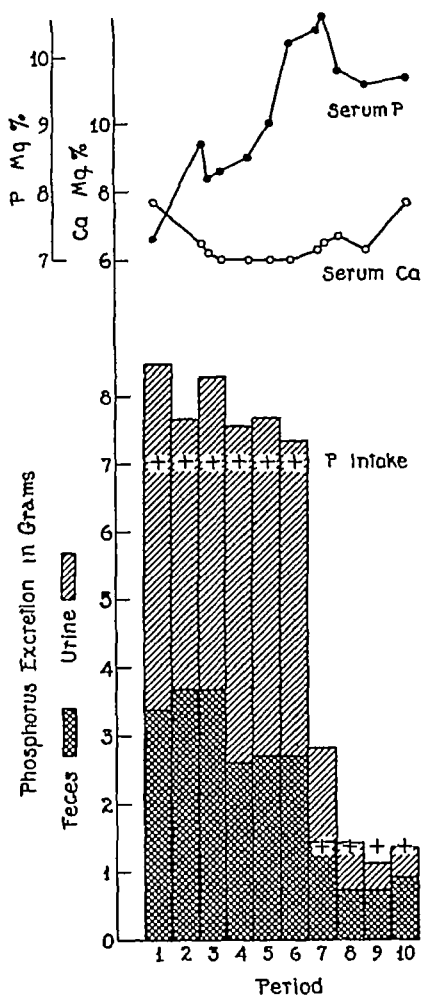
CHART 3 GRAPHIC REPRESENTATION OF PHOSPHORUS METABOLISM IN
EXPERIMENT 3

for calcium and phosphorus [3] Furthermore, after period 1, because of certain marked discrepancies which were at once noted, the day urine (7 a m to 7 p m) was collected and analyzed separately from the night urine   The results are shown in table 3 and chart 3

Results   Before drawing any conclusions regarding the phosphorus balance, we might have made allowance for the phosphorus deposited with the protein in muscle and with the calcium in bones (11)   However, examination of the calcium and nitrogen balances shows that these factors are smaller than the probable errors of the investigation Also, it is true that too much reliance cannot be placed upon balance columns where very high intakes are involved, because a small percentage error in the intake would make a considerable error in the balance   Even so, although the serum phosphorus did rise considerably, it is quite apparent from the phosphorus balances (table 3) that, during the first six periods, while on a high phosphorus intake, the patient's kidneys had little difficulty in excreting phosphorus Furthermore, when the phosphorus intake was suddenly lowered the excretion of urinary phosphorus fell immediately rather than slowly, as it would, had there been any previous difficulty in excreting phosphorus

The marked polyuria at night (table 3) was very striking, especially during the first six periods   It is also of interest that there was a relative increase in the urinary excretion of calcium, phosphorus, and nitrogen at night proportional to the increased excretion of water Like the polyuria noted in experiment II in the first hour following parathormone injection, these will have to remain as isolated observations for the time being   They lead one to suspect some abnormality in the water balance

Comment   7 At any given level of hypoparathyroidism there was no marked difficulty in excreting phosphorus nor could the blood chemistry be markedly altered by high and low phosphorus diets

DISCUSSION

The serum calcium and serum phosphorus curves in experiment I certainly suggest that one ion rises because the other falls or vice

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[3] We are much indebted to Dr Joseph C Aub and his associates at the Massachusetts General Hospital for having the feces analyzed for us

versa   One cannot escape the idea that this relationship is dependent on the ability of the serum to hold calcium phosphate, and that, in the presence of normal bones, the blood calcium contains all the calcium and phosphorus ions which its physical state will permit   It also seems almost certain that the increased phosphorus excretion, the increased calcium excretion, the decreased serum phosphorus, and the increased serum calcium following parathormone administration are four interrelated facts

Our work, together with that of Albright, Bauer, Ropes, and Aub (3) suggests the following tentative hypothesis as to the modus operandi

When parathormone is administered, the equilibria of the body fluids are upset in such a way that an increased phosphorus excretion is a necessary result   We do not know the cause of the increased phosphorus excretion, but as a result of this increased phosphorus excretion the body fluids become depleted in phosphorus   The falling serum phosphorus is evidence of this   As the phosphorus and consequently the phosphate ions in the serum fall, there is a tendency to an unsaturation of the blood with calcium phosphate   This tendency is met by a mobilization of calcium phosphate from the bones   Thus a deficit in phosphate ions is being supplied by calcium and phosphate ions.   Consequently the serum calcium rises   With a rise in the serum calcium, provided the level is not below the threshold for calcium excretion, there is a rise in urinary calcium output

The sequence of events which takes place after parathormone administration (chart 1) strongly supports such an hypothesis, except that we have been unable to show consistently that the fall in serum phosphorus precedes, by any detectable interval, the rise in serum calcium

It may be that the calcium phosphorus equilibrium is so finely adjusted that we have not been able to detect the small initial change, or possibly we have not yet studied short enough periods

On the basis of our hypothesis, the question immediately arises as to what causes the increased phosphorus excretion after parathormone injection   It seemed at first that there might be a specific impairment of renal function in hypoparathyroidism whereby phosphorus excretion was inhibited   However, in experiment III, the

high phosphorus excretion during a high phosphorus diet and the immediate fall to a low phosphorus excretion when the diet was changed to one low in phosphorus would seem to disprove this   All that we can say, therefore, is that some change in the blood equilibria occurs as the result of parathormone administration which makes necessary this increased phosphorus excretion

<div align="center">SUMMARY AND CONCLUSIONS</div>

1   The clinical aspects of a case of idiopathic hypoparathyroidism are reported

2   The criteria on which the diagnosis of idiopathic hypoparathyroidism is made are

a   Low serum calcium

b   High serum phosphorus

c   Cataract

d   Normal density of bones by x-ray

e   Aggravation of tetany by exertion

3   Following the injection of an active parathyroid extract, an increase in phosphorus excretion was immediately detectable and reached its maximum within the first two hours   The mechanism of this is not apparent from our data

4   Evidence is educed from study of the metabolism of the patient to show that the increase in serum calcium, the increase in calcium excretion, and the decrease in serum phosphorus following parathormone administration may all be the sequelae of this increase in phosphorus excretion

5   An hypothesis is suggested to explain these interrelationships

6   By using high and low phosphorus diets, it seems that there is no inability to excrete phosphorus in hypoparathyroidism and it follows, therefore, that the increased excretion of phosphorus following injection of parathormone is not due to an increase in the excretory ability of the kidney for this element

7   As the serum calcium rose from 5 2 to 11 2 mgm per 100 cc following the injection of parathormone there was a critical serum calcium value of about 8 5, at which point an almost negligible urinary calcium excretion suddenly changed to a very appreciable one   When the serum calcium was above 8 5 the urinary calcium increased as the

serum calcium rose then decreased abruptly as soon as the serum calcium fell below 8 5 mgm per 100 cc   This suggests that there is a threshold for urinary calcium excretion and that this threshold is below the normal value for serum calcium

We wish to express our gratitude to Miss M Struve for her assistance in arranging diets and superintending the collection of specimens.

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THE DETERMINATION OF THE CIRCULATING BLOOD VOLUME IN INFANTS BY THE CARBON MONOXIDE METHOD

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The carbon monoxide method for determination of blood volume has been used with success in experiments on adults, but has to our knowledge not been previously applied to infants   Miller (1) in 1910 employed the procedure as refined by Zuntz and Plesch (2) in studying a group of thirty children ranging from 6 to 16 years of age, but no reports of studies in younger individuals are available

The principle of this method was first advocated by Gréhant and Quinquaud (3), and was first applied to the study of blood volume in man by Haldane and Lorrain Smith (4)   Improvements in the form of application have been contributed by a number of workers (5), particularly by Salvesen (6) and by Harrop and his associates (7) An accurately measured quantity of carbon monoxide gas is delivered into a breathing apparatus, from which it is absorbed rapidly from the respired air   The time required for maximal absorption, and the conditions under which absorption is accelerated, have been carefully determined by Harrop   After a suitable interval has elapsed for the establishment of equilibrium between the gas in the circulating blood and the respired air, and for complete mixing of the blood, a sample of the latter is withdrawn from a vein and analyzed for carbon monoxide content by an adaptation of the method of Van Slyke and Robscheit Robbins (8)   When the proper corrections have been applied, the calculation of the circulating blood is then simplified to the following equation

$$\text{Circulating blood volume} = \frac{\text{Quantity of gas absorbed} \times 100}{\text{Percentage of gas in the blood}} \qquad (1)$$

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TECHNIQUE

Pure carbon monoxide is generated from a mixture of formic acid and sulfuric acid with the application of heat, is washed in a trap containing potassium hydroxide solution to remove traces of free acid, and stored in bottles over a mixture of equal parts of glycerine and saturated solution of sodium chloride, a mixture chosen by Harington and Van Slyke (9) for its low vapor tension and low gas solubility Samples of the stock of gas are analyzed in the Harington-Van Slyke
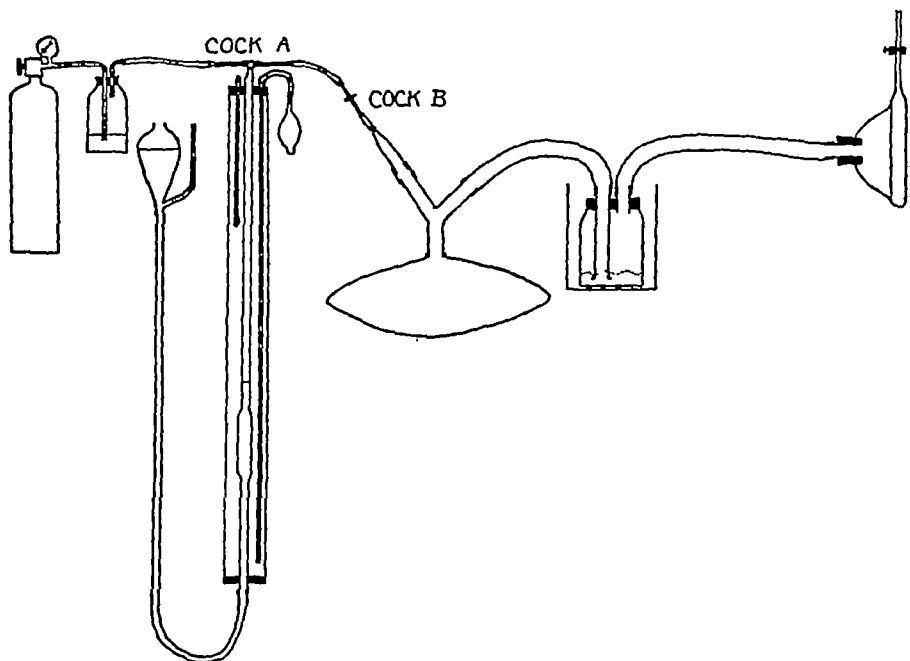
FIG 1 DIAGRAM OF APPARATUS FOR ADMINISTRATION OF A MEASURED AMOUNT OF CARBON MONOXIDE

manometric apparatus to determine the purity factor, the results of parallel determinations agreeing usually within 0 2 per cent In clinical tests the gas is measured from a specially constructed buret, fitted with a three-way capillary stopcock (cock *A* in fig 1) at the top, and provided with a water jacket, thermometer, and levelling bulb filled with glycerol-salt mixture, adapted for delivering any quantity of gas up to about 27 cc with an error of measurement of probably less than 0 05 cc

The breathing apparatus consists of a face-mask, a soda-lime jar, and a rubber balloon, connected by rubber tubing. The face-mask is made of a half-ovoid of transparent celluloid fitted with a rubber pneumatic cushion about the rim and a tight fitting one hole rubber stopper piercing the apex of the dome. The mask is connected to the soda lime jar by a piece of rubber tubing of 17 mm bore. The soda-lime jar is made from a pint mason jar and two test tubes, fixed in place with plaster of paris and sealed with sealing wax. To facilitate changing the soda lime the jar has been broken along a circle about 2 cm from the bottom, so that it may be taken apart, and sealed up with soap and adhesive tape, reinforced with two strong wide rubber bands cut from an inner tube. No 4 mesh soda-lime is used, held in place by two circular pieces of fine wire gauze, one at the bottom and one at the top of the jar, and is changed after every one or two determinations. During tests on patients the soda lime jar is immersed in a jacket containing ice water to lower the water vapor tension of the rebreathed air and diminish the discomfort of breathing warm air, and to control leakage. A piece of rubber tubing of 17 mm bore connects the soda-lime jar with a large-bore glass Y-tube, to one shank of which is attached a football bladder, and to the other, suitable rubber and glass tubing leading to a one-way stop-cock (cock B). All connections are wired or sealed with sealing-wax

In testing for leaks, the buret was connected to the breathing apparatus by a piece of small bore rubber tubing joining cocks A and B, and the face-mask was tied firmly against a greased porcelain plate so that the pneumatic cushion was in apposition throughout its circumference. A positive pressure of about 30 cm of glycerol-salt solution was then exerted on the air in the gas buret, and with the levelling bulb clamped in this position the loss of gas pressure was noted. When the leakage amounted to a pressure change of less than 0 15 mm of glycerol salt per minute, the apparatus was considered suitable for use in clinical tests. During actual use, leakage through the division in the soda lime jar is prevented by immersing it in water to a depth of about 10 cm, since the balloon is never allowed to become sufficiently distended, during a test, to put the rubber on the stretch, it is not possible for the air contained in the breathing system to overcome the critical pressure exerted by this protective water-jacket

Of course, it has not been possible to make control tests of leakage around the edges of the mask when applied to a patient, but this source of error is minimized as far as possible by the liberal application of petrolatum to the pneumatic cushion, by the constant application of firm manual pressure to the mask, and by careful attention to this part of the apparatus throughout the breathing period   In preliminary tests with the mask, which are always made with each patient before carbon monoxide is let into the system, different positions of the mask are tried out until a good apposition is obtained, which can then be held until the end of the experiment

Before a determination, the gas buret is filled to a convenient level with carbon monoxide and the levelling bulb is clamped in a position causing the contents of the buret to be at a pressure less than atmospheric, at least fifteen minutes are allowed for drainage of solution from the walls of the buret before the pressure is brought to atmospheric and the volume, temperature, and barometric pressure recorded A small oxygen tank fitted with a water trap and pressure gauge is then connected to the gas buret by one arm of cock A The other arm is connected to cock B The contents of the balloon are expelled, and the tube connecting the face-mask and the soda-lime jar clamped off   With cock A turned so as to close the buret but allow communication between the oxygen tank and the breathing apparatus, oxygen is run into the balloon in an amount estimated to be about four or five times the tidal air of the patient   A sample of blood, usually 7 cc to permit duplicate determinations, is now drawn from the patient under paraffin oil and delivered under oil into a bottle containing oxalate   Even in small infants a definite quantity of gas absorbable by Winkler's solution is usually detectable in these preliminary samples, and is probably either evidence of the known contamination of urban atmosphere with carbon monoxide derived from illuminating gas, automobile exhaust gas, and from other sources, or represents a systematic error of the method   The mask, with the pneumatic cushion well greased, is then applied to the patient's face, the clamp removed from the tube connecting the mask with the soda-lime jar, and the patient allowed to breathe into the apparatus for a short period during which the position of the mask is adjusted for the best approximation   With the levelling bulb of the gas buret raised to the

level of cock *A*, and with cock *B* open, carbon monoxide is delivered into the breathing apparatus in an amount estimated to produce a final concentration in the patient's blood of approximately 3 volumes per cent Cock *A* is then turned so that the buret is closed but the connection between the oxygen tank and the breathing apparatus open, and a slow stream of oxygen allowed to run in at a rate which will keep the balloon nearly empty at full inspiration A breathing period of ten minutes or more is allowed, at the end of which a second sample of blood is withdrawn under oil while the mask is still in place.

TABLE 1

Factors by which P_{CO} is multiplied to calculate volume per cent of CO

Temperature	Factor when P_{CO} is measured with gas at 0.5 cc. volume
°C	
20	0 02059
21	0 02050
22	0 02042
23	0 02035
24	0 02028
25	0 02021
26	0 02014
27	0 02007
28	0 02000
29	0 01993
30	0 01986

In the equation of Van Slyke and Neill, $a = 0 5$
$$S = 15 0$$
$$i = 1 00$$
$$\text{cc. sample} = 3 0$$

The oxygen stream is then turned off, cock *B* closed, the mask clamped off and removed The analysis of the blood samples for carbon monoxide is made in duplicate on 3 cc portions by an adaptation of the method of Van Slyke and Robscheit-Robbins (8), and the percentage of added carbon monoxide is then determined by subtraction of the average figures The factors used to convert differential pressure readings to volumes per cent of gas are calculated from the equation of Van Slyke and Neill (10) and are given in table 1

The air resistance in the apparatus so designed is not great, and is

compatible with comfortable rebreathing during periods as long as twenty minutes when tests are made on older children whose confidence can be gained   Most of the infants, naturally, have fretted or cried during the greater part of the breathing period, though a few have slept throughout   Since the rapidity with which the gas is absorbed depends to a large extent on the rate of pulmonary ventilation, it has been customary to prolong the breathing period an additional five or ten minutes in the case of infants who remain quiet With vigorous crying some of the infants become slightly cyanotic, but not more so than is commonly seen under ordinary circumstances In a few instances where toxic infants were being studied the color improved as a result of the administration of oxygen incidental to the test   We have observed no harmful effects attributable either to the carbon monoxide specifically or to the test in general, and the disadvantages to the patient are apparently confined to the loss of about 15 cc of blood and the discomfort of the two venapunctures and temporary restriction of activity   The gas does not appear to be retained long, in one patient in whom the test was repeated after 46 hours, the concentration of carbon monoxide had fallen from a level of 3 75 volumes per cent at the termination of the first test to 0 11 volumes per cent at the commencement of the second   Since carboxyhemoglobin does not participate in the oxygen-carrying function of blood, it is obvious that the carbon monoxide method of blood volume determination should not be applied to patients with severe anemia

DISCUSSION OF ERRORS

1 The denominator in equation I   The analytical error in duplicate determinations by the foregoing technique depends very little on the amount of carbon monoxide present in the sample of blood In a series of 13 duplicate analyses of samples drawn before any administration of carbon monoxide, the average deviation from the mean in the different pairs of determinations, expressed in volumes per cent of the gas at N T P , was 0 014 ±0 012[1] volumes per cent   In another series of 30 duplicate analyses after carbon monoxide had been given for blood volume determinations, where the final concentration

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[1] The second figure indicates the standard deviation

was somewhere between 1 4 and 5 3 volumes per cent, the error expressed in the same way was 0 025 ±0 018   Thus, the analytical error may be expressed as follows

$$\text{Analytical Error} = \frac{\text{Sum of absolute errors for both samples} \times 100}{\text{Difference of the mean of the two samples}}$$

$$= \frac{(0\ 014 \pm 0\ 012 + 0\ 025 \pm 0\ 018) \times 100}{3\ 00}$$

or approximately 2 3 per cent for an elevation of the carbon monoxide level of 3 00 volumes per cent.   For a larger amount of gas administered, the error is correspondingly diminished

2 The numerator in equation I   The measurement of the quantity of carbon monoxide absorbed by the patient involves three sources of error   (a) determination of the amount of gas injected into the breathing system, (b) leakage from the system during the breathing period, and (c) determination of the residue of carbon monoxide remaining in the apparatus at the end of the breathing period

As a matter of convenience precautions are taken to prepare fairly pure carbon monoxide gas by washing out the generator and the storage bottles several times before the final collection of gas for stock. The advantage of making the tests for purity in the Harrington-Van Slyke manometric apparatus is twofold   in the first place, satisfactory checks are readily obtained, and in the second place, the use of Winkler's solution for the final absorption of the gas, by an identical procedure to that used in blood analysis, offers a better basis for comparison of results than other methods such as combustion. As was stated previously, the results of duplicate determinations usually agree within 0 2 per cent   The measurement of the gas delivered into the breathing apparatus from the gas buret is held to be accurate to 0 05 cc , including herein the errors associated with barometer and thermometer readings, so that for a volume of 20 cc at room temperature the error from this source amounts to less than 0 25 per cent.

A preliminary test was made to determine the rate of diffusion of carbon monoxide through rubber   Under a positive pressure of about 30 cm of glycerol salt solution, the pure gas passes through the wall

of an ordinary piece of thin-walled rubber tubing at a rate of approximately 0 0003 cc per square centimeter of surface per hour At the low partial pressure of CO in the apparatus during a blood volume determination, this leakage does not introduce an appreciable error

The control of leaks in the breathing apparatus has been dealt with to some extent previously After every two or three determinations the entire apparatus is tested by immersion in water and exertion of positive pressure on the balloon It is always possible by the use of sufficient force to cause bubbles to appear around the break in the

TABLE 2

Duplicate determinations in individual patients with short time intervals

Patient	Date	Weight	Blood volume	Blood volume	Plasma volume	Plasma volume
		kgm	*cc*	*cc per kgm*	*cc*	*cc per kgm*
A H	April 9, 1928	7 29	620	85 0	370	50 8
	April 27, 1928	7 58	560	75 2	290	38 6
M C	May 17, 1928	8 30	600	72 3	337	40 6
	May 19, 1928	8 27	565	68 3	323	39 1
M A	September 4, 1928	4 72	320	67 8	196	41 5
	September 14, 1928	4 81	320	66 5	185	38 5
F B	September 10, 1928	4 30	270	62 8	164	38 1
	September 19, 1928	4 42	300	67 9	198	44 8
L G	September 15, 1928	6 10	355	58 2	218	35 7
	September 26, 1928	6 38	355	55 6	199	31 2

soda-lime jar, but, as was also stated above, this part of the apparatus is sealed off by a water trap during tests on patients The most obvious source of error in respect to leakage is, of course, the face-mask, to the careful approximation of which the greatest attention is given during the entire breathing period Experimental control of this factor by duplicate determinations of blood volume on individual patients predicates a basic assumption of the constancy of the blood volume which, in the light of present knowledge, must be considered still an open question Five such duplicate tests, performed with intervals of

from 2 to 18 days, are listed in table 2 and suggest, at any rate, that the error here is at least not large

The quantity of carbon monoxide absorbed by the patient is the difference between the amount injected into the breathing apparatus and the amount remaining in it at the end of the breathing period Such residual amounts are small but detectable, and while in our determinations they have not been found to be sufficiently great to influence the final figure for total blood volume, as calculated without taking them into account, by more than 2 7 per cent, and therefore within the total error of the method, it is possible that one may be able at some time to show that in the presence of acidosis they will be large enough to be important, as suggested by the work of Stadie (11) on the absorption coefficient of blood for carbon monoxide under varying hydrogen-ion concentrations   Salvesen (6) in determining the blood volume of rabbits by the carbon monoxide method, had made use of sheep's blood as a reagent for absorbing the residual gas, determining the absorption coefficient of this particular blood by exposing samples of it to varying atmospheric concentrations of carbon monoxide   In our first attempts to utilize this method, with samples of human blood as the absorbing reagent, we were unable to detect the presence of any residual carbon monoxide by simple exposure of the residual air in the apparatus to the blood used   However, since it has been shown that the dissociation curve of carbon monoxide hemoglobin exposed to an atmosphere containing both oxygen and CO is expressed by the equation

$$\frac{HbCO}{HbO_2} = K \times \frac{(CO\ percentage\ in\ air)}{(O_2\ percentage\ in\ air)} \tag{II}$$

it seemed likely that this analytical method might be considerably refined by removing the oxygen from the residual air before exposing it to the blood used for absorption of the carbon monoxide

The method used is similar to that employed by Whipple and his associates (12), except that it requires no special apparatus other than two or three Haldane gas sampling tubes   The volume of residual air is first determined by adding together the contents of the balloon, of the breathing apparatus (exclusive of the balloon) from cock B to the pneumatic cushion of the mask, and of the "dead space" of the

of an ordinary piece of thin-walled rubber tubing at a rate of approximately 0 0003 cc per square centimeter of surface per hour At the low partial pressure of CO in the apparatus during a blood volume determination, this leakage does not introduce an appreciable error

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patient was suffering from tuberculous meningitis, and during the rebreathing period remained on the whole more quiet than the average patient.

In order to compare the results of determinations of blood volume by the carbon monoxide method with those obtained with the dye method, simultaneous tests were performed on ten patients using both methods in the following way   A preliminary control experiment was made to determine whether the addition of brilliant vital red, the dye used, to human blood would interfere with subsequent absorption of carbon monoxide   Ten cubic centimeters of oxalated human blood were placed in each of two beakers of equal size, and to one sample was added 0 05 cc of physiological salt solution, and to the other 0 05 cc

TABLE 5

Influence of presence of brilliant vital red in blood on absorption of CO

Experiment number	CO content of control blood (saline added)		CO content of test blood (dye added)		Difference (control—dye)
	Duplicate analyses	Average	Duplicate analyses	Average	
	volumes per cent	*volumes per cent*	*volumes per cent*	*volumes per cent*	*volumes per cent*
1	1 027 1 051	1 04	0 924 0 923	0 92	0 12
2	3 776 3 742	3 76	3 518 3 531	3 52	0 24

of a 1 per cent solution of the dye in order to give it a final concentration approximately the same as that effected by the use of the dye in clinical tests   Both beakers were then placed in a vacuum desiccator equidistant from the inlet tube, and with the desiccator lid in place, but not tightly sealed, approximately 100 cc of carbon monoxide gas were slowly admitted, displacing some of the contained air   The lid was then sealed and the desiccator gently agitated for 15 minutes to permit the gases to attain equilibrium with the blood samples   On removal of the lid, a layer of paraffin oil, one or two centimeters deep, was added to the contents of each beaker, and the two samples were then analyzed for carbon monoxide content   The results, shown in table 5, agreed within the error of the method, we believe, at any

always in the same direction in that disregarding the correction causes a false high blood volume figure In comparative determinations on the same patient this is relatively unimportant

Chang and Harrop (7) found that in the case of adults at rest and breathing quietly, practically complete absorption of the carbon monoxide injected into the breathing apparatus had taken place after 15 minutes of rebreathing It seemed probable that this interval

TABLE 3

Absorption of carbon monoxide as determined by analysis of residual air

Patient	CO measured from buret	Unabsorbed CO by analysis	Per cent CO absorbed
	cc at N T.P	*cc at N T.P*	*per cent*
S F	11 450	0 422	96 31
J L	21 340	0 536	97 49
D M	12 925	0 258	98 00
M S	14 945	0 100	99 34
L G	14 170	0 071	99 50
R S	19 845	0 035	99 82

TABLE 4

Control of time required for absorption of CO
Blood carbon monoxide content

	At start	After 5 minutes	After 10 minutes	After 15 minutes
	volumes per cent	*volumes per cent*	*volumes per cent*	*volumes per cent*
A B	0 077		3 43	3 30
C L	0 081	2 88	2 89	
R S	0 066	4 15	4 22	
L S	0 077	5 12	5 27	

might well be shorter in the case of infants crying vigorously, and the choice of a ten minute breathing period in our technique is based on a small number of control observations which bore out this supposition, and which are given in table 4 This series has not been enlarged on account of the undesirability of removing additional blood, but the results in patient R S , where this control was supplemented by analysis of the residual air for unabsorbed carbon monoxide, show that the ten minute period is, in all probability, adequate This

patient was suffering from tuberculous meningitis, and during the rebreathing period remained on the whole more quiet than the average patient.

In order to compare the results of determinations of blood volume by the carbon monoxide method with those obtained with the dye method, simultaneous tests were performed on ten patients using both methods in the following way   A preliminary control experiment was made to determine whether the addition of brilliant vital red, the dye used, to human blood would interfere with subsequent absorption of carbon monoxide   Ten cubic centimeters of oxalated human blood were placed in each of two beakers of equal size, and to one sample was added 0 05 cc of physiological salt solution, and to the other 0 05 cc

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rate, the error occurred in a direction which would have given, if anything, an abnormally high blood volume by the carbon monoxide method

In the experiments with patients tabulated in table 6, the dye method of Keith, Rowntree, and Geraghty (13) was used, slightly modified to suit the conditions of work with infants Either 1 00

TABLE 6

Comparison of CO method and dye method in simultaneous blood volume determinations in 10 patients

Patients	Weight	Age	Blood volume (CO method)	Blood volume per kilo gram (CO method)	Blood volume (dye method)	Blood volume per kilo-gram (dye method)	B V (CO) − B V (dye)	$\frac{B V (CO) - B V (dye)}{Weight}$
	kgm	months	cc	cc per kgm	cc	cc. per kgm	cc	cc per kgm
L S	3 53	12	275	77 9	595	168 6	−320	−90 7
F B	4 42	2	300	67 9	400	90 5	−100	−22 6
R W	5 68	7	445	78 3	520	91 5	−75	−13 2
S E	6 72	5	410	61 0	720	107 1	−310	−46 1
J L	6 75	12	500	74 1	475	70 4	+25	+3 7
D B	6 87	8	440	64 1	500	72 8	−60	−8 7
W P	6 90	15	455	66 0	845	122 5	−390	−56 5
R S	7 47	11	500	66 9	550	73 6	−50	−6 7
C L	7 80	17	605	77 6	455	58 3	+150	+19 3
J J	8 22	21	655	79 6	635	77 2	+20	+2 4
Arithmetical mean				71 ±2		93 ±10		
Standard deviation				6 6 ±1 5		31 ± 7		
Coefficient of variation				9 ±2		33 ± 7		

1 50 or 2 00 cc of a 1 per cent solution of brilliant vital red, diluted to approximately 10 cc with physiological salt solution, was injected, depending on the size of the patient The same graduated 2 cc pipet was used to measure the dye to be injected and to make up the standard dilution of dye for use in the colorimeter For the first sample of blood, 10 cc were removed, of which 4 cc were oxalated under oil

for a single determination of the carbon monoxide content and for hematocrit tests, the rest being allowed to clot in a centrifuge tube for control of the dye method   The dye was then injected, and four or five minutes later the second sample of blood was drawn, 5 or 6 cc in amount, and either oxalated or allowed to clot in a centrifuge tube. A measured amount of carbon monoxide gas was then administered according to the technique outlined previously, and at the end of the usual breathing period 7 or 8 cc of blood were removed and oxalated under oil   In calculating the total blood volume for comparison with the two methods, the figures were corrected by the addition of the amount of blood previously withdrawn   The disagreement between the results of the two methods was often pronounced, and was neither of constant magnitude nor of uniform direction   In seven out of ten such determinations the value of total blood volume expressed in cubic centimeters per kilogram of body weight was higher with the dye method than when the carbon monoxide method was used, and the average of all the determinations with the dye method was 93 cc per kilogram, with the carbon monoxide method, 71 cc per kilogram The largest discrepancy between the two methods occurred in one patient in whom the blood volume as determined by the dye method exceeded that obtained by the gas method by 320 cc., or 91 cc per kilogram, while, in the other direction, the dye method in another patient gave a result that was lower by 150 cc or 19 cc per kilogram than the value given by the carbon monoxide method   If, as has been suggested by previous workers with both methods, the body weight is in any sense a criterion for the expected blood volume, it is clear from these figures that the carbon monoxide is in our hands the more reliable   Perhaps the obvious conclusion one might wish to draw is that our technique with the dye method is faulty, but we are not convinced that this is a complete explanation of the discrepancy Whipple and his associates (14) in a comparison of the dye, carbon monoxide, and the exsanguination methods for determining total blood volume in dogs, likewise found discrepancies of similar magnitude and direction   In all but one of fourteen dogs the value of total blood volume expressed in cubic centimeters per kilogram of body weight was higher with the dye method than when the carbon mon oxide method was used, and the average of all the determinations

for the dye method was 103 9 cc per kilogram, for the carbon mon-
oxide method, 86 9 cc per kilogram   It is not within the scope of
this paper to discuss the theoretical aspects of the application of these
different methods, and we do not at the moment contend that the
carbon monoxide method as described here eludes all the theoretical
pitfalls of clinical determination of the total blood volume, at the
same time, it has in our hands exhibited a closer correlation between
blood volume and body weight, as expressed in the coefficient of
variation, than have determinations made by the dye method, and
there is considerable experimental evidence in the literature that the
true blood volume follows body weight in health fairly closely, pro-
vided suitable allowances are made for conditions of obesity or severe
undernutrition

REVIEW OF PREVIOUS WORK IN BLOOD VOLUME OF INFANTS

In this discussion we shall not include the question of the blood
volume of newly born infants, since our material does not cover this
age group   The investigations of Lucas and Dearing (15) are unique
in this field

The authors just mentioned, in a series of 30 estimations made on
11 nearly normal infants varying in age from two weeks to a year,
found an average quantity of 110 cc of blood per kilogram of body
weight, as determined by the dye method   Individual patients,
though their blood volume might deviate considerably from the
average figure when calculated with relation to body weight, never-
theless tended to maintain a fairly constant ratio over a period of as
long as two months, which was the maximum interval covered by the
duplicate determinations   According to these authors, the ratio of
blood volume to body weight is more constant than the ratio plasma
volume to body weight   We have calculated the standard deviation
and the coefficient of variation[2] of this series and obtain for the latter
the figure 9 5

[2] The formulas for the calculation of these functions are as follows

m_1 = a single observation (such as a determination of B V /Wt)

n = number of observations

M = arithmetical mean of all observations of the type m_1

$$= \frac{\Sigma\, m_1}{n}$$

Bakwin and Rivkin (16), also using the dye method, determined the blood volume of 36 normal infants ranging in age from 12 days to 10 months, and obtained an average ratio of 101 cc per kilogram of body weight   In this series the standard deviation was 14 6 and the coefficient of variation 14   Plasma volumes averaged 61 cc per kilogram for the same group, and here the coefficient of variation was 12, indicating a slightly closer correlation than blood volume had shown

Marriott and Perkins (17) in a study of 7 approximately normal infants under one year of age obtained with the dye method an average figure of 9 1 per cent of the body weight (which may mean 91 cc per kilogram if their figure refers to volume of blood and not mass), with variations from 8 0 to 10 8 per cent   Their individual determinations were not reported

Darrow, Soule, and Buckman (18) demonstrated a closer agreement between plasma volume and weight than between blood volume and weight   They showed that for any particular age group a practically identical degree of correlation exists between plasma volume per kilogram and plasma volume per square meter of body surface, and further that the part which age plays is expressed in the fact that the expected plasma volume per kilogram rises from an initial level of 50 cc. per kilogram shortly after birth (calculated from the observations of authors previously quoted) to a level of about 62 cc during the first year of life, subsequently falling slowly to a normal level of 50 cc during the fourth year, where it remains through the rest of childhood, or up to 11 years of age which was the upper limit of their study   They found that the ratio plasma volume to body weight varied more widely during the first year of life than later   The curve of variation with age of the ratio plasma volume per square meter of body surface was more complicated, showing a rounded peak at one year followed by a gradual rise throughout childhood, and in general the ratio blood volume per square meter followed a parallel curve.

Standard Deviation $= \sqrt{\dfrac{\Sigma(m_1 - M)^2}{n}}$

Coefficient of Variation $= \dfrac{100 \times \text{Standard Deviation}}{M}$

They calculated the coefficients of variation (referred to by them as the percentage standard deviation) of the ratio plasma volume per kilogram separately for each year of life during the first 11 years and obtained figures ranging from 2 to 8   Such close agreement as these low coefficients express indicates that in average normal children the

TABLE 7

Twenty-four blood volume and plasma volume determinations in 22 patients

Patient	Blood volume	Plasma volume	Age	Weight	Length	Stem length	Surface area (Du Bois)	Surface area (Mech)	Surface area (Howland)	Clinical condition for which patient was admitted
	cc	cc	mos	kgm	cm	cm	sq cm	sq cm	sq cm	
J G	220	136	1	2 18	51 5	33 5	1,740	2,001	1,785	Congenital syphilis
L S	275	171	12	3 53	59 5	39 5	2,375	2,759	2,435	Congenital syphilis
D M	290	190	2	3 55	55 5	37 5	2,265	2,769	2,445	Nutritional disturbance
F B	300	198	2	4 42	58 0	37 0	2,567	3,205	2,865	Nutritional disturbance
F T	320	172	5	4 58	64 0	40 5	2,800	3,282	2,942	Malformation of spine
M A	320	185	6	4 81	63 0	40 0	3,100	3,390	3,053	Nutritional disturbance
J W	340	225	2	4 36	57 0		2,520	3,175	2,836	Otitis media
E E	345	209	4	3 68	59 0	39 5	2,412	2,836	2,507	Mongolian idiocy
L G	355	199	9	6 38	70 0	43 5	3,440	4,093	3,811	Pneumonia
C K.	370	272	3	3 30	54 0	35 5	2,160	2,638	2,324	Prematurity
L H	385	232	5	5 20				3,571	3,241	Pneumonia
S E	410	212	5	6 72	72 5	40 3	3,610	4,238	3,976	Nutritional disturbance
D B	440	304	8	6 87	65 0	40 3	3,370	4,300	4,049	Pneumonia
R W	445	315	7	5 68	74 0	41 5	3,403	3,788	3,474	Congenital syphilis
W P	455	263	15	6 90	75 0	47 0	3,736	4,312	4,062	Congenital cataract
J L	500	270	12	6 75	72 0	43 0	3,589	4,250	3,990	Pneumonia, empyema
A B	505	219	15	7 63	75 0	49 5	3,900	4,611	4,415	Nutritional disturbance
J P	535	298	13	6 35				4,080	3,797	Pertussis, pneumonia
M K.	550	332	9	5 73	67 5	45 0	3,190	3,811	3,498	Pyuria
A H	560	291	23	7 58	77 0		3,966	4,592	4,391	Pertussis, encephalitis
M C	565	323	11	8 27	73 0		3,950	4,867	4,724	Scarlatina, mastoiditis
M C	600	337	11	8 30	73 0		3,960	4,878	4,739	Scarlatina, mastoiditis
A H.	620	370	22	7 29	77 0		3,900	4,473	4,251	Pertussis, encephalitis
J J	655	367	21	8 22	76 0	47 5	4,070	4,847	4,700	Pertussis, pneumonia

body weight is more than a fair index of the total plasma volume as determined by the dye method   It is further suggested by these authors that plasma volume appears to be related primarily to metabolic rate, and that its relations to body weight and surface area are of secondary degree and subject to the same limitations as govern the

estimation of metabolic rate from measurements of body mass and area

EXPERIMENTAL RESULTS

Table 7 presents the results of 24 determinations of blood volume in 22 hospital patients admitted for a variety of causes and who, at the time the determination was made, were not thought to be suffering from any circumstance that would bring about a temporary alteration of blood volume   In other words, the figure obtained for each patient was considered to represent a normal level for that individual at that particular time   Only the age group of the first two years is included As a measure of the normal blood volume at this age they, of course, suffer the defect of not comprising any strictly normal individuals, yet conversely, by including a variety of nutritional states they are possibly better adapted for showing what correlations are most tenaciously held when the individual deviates widely from the average, and may thus serve to demonstrate, for example, whether body weight or surface area is a measure of total blood volume, even better than would an assembly of entirely normal values

In comparison of our results with those obtained by others with the dye method, the fundamental differences between the two methods must be borne in mind   first, that the dye method measures the plasma volume directly   while the carbon monoxide method measures the circulating blood volume directly, and secondly, that in the former method the added substance is mixed entirely or mainly in the plasma, whereas in the latter the gas is taken up almost exclusively by the red cells   According to Whipple and his associates (14), these differences are largely responsible for the higher figures which so many observers have obtained in working with the dye method, and in their hands the carbon monoxide method has given in dogs a higher figure than is yielded by the exsanguination method, though it closely approximates the results of the bleeding method where the latter is combined with procedures adapted for including in the total blood volume a figure calculated from the hemoglobin derived from extraction of tissues—in other words, when the non-circulating hemoglobin is included in the total blood volume figure   We do not agree with the statement made by Darrow, Soule, and Buckman (18) that the carbon monoxide method gives results that are lower than the actual blood volume

In table 7 we have listed the determined values for blood volume and plasma volume, together with the age, body weight, length, stem length, and body surface as calculated by the three different formulae of Du Bois, Meeh, and Howland (19)   The coefficients of correlation (20) in table 8 express the degree of agreement between observations of blood volume or plasma volume and the corresponding measurement of the function under consideration, in the sense that no agreement at all is indicated by a coefficient of 0, perfect agreement by a coefficient of ±1   For a more detailed discussion of the significance of coefficients of correlation and variation, one is referred to appropriate

TABLE 8

Coefficients of correlation of blood and plasma volume with various body measurements

	Blood volume	Plasma volume
Age	0 79 ±0 08*	0 63 ±0 12
Body weight	0 89 ±0 05	0 72 ±0 11
Body length	0 86 ±0 06	0 68 ±0 11
(Body length)²	0 86 ±0 06	0 68 ±0 11
(Body length)³	0 86 ±0 06	0 68 ±0 11
Stem length	0 73 ±0 11	0 50 ±0 18
(Stem length)²	0 74 ±0 11	0 49 ±0 18
(Stem length)³	0 73 ±0 11	0 48 ±0 19
Surface area (Du Bois formula)	0 89 ±0 05	0 71 ±0 11
Surface area (Meeh-Rubner-Heubner formula)	0 87 ±0 05	0 72 ±0 10
Surface area (Howland formula)	0 88 ±0 05	0 73 ±0 10

* The second figure in each column represents the standard error of the coefficient r, obtained from the formula $e(r) = \dfrac{1 - r^2}{\sqrt{n}}$

treatises on the theory of statistics, in general, however, there is a parallelism in the degree with which the former approaches ±1 and the latter approaches 0 when correlation of the two variables is treated by the two methods

The coefficients of correlation listed in table 8 show that the linear correlation is best between blood volume and body weight, blood volume and surface area, and blood volume and body length   On the other hand plasma volume shows poor agreement with any of these measurements   If the blood volumes are plotted as ordinates against the body weight as abscissae, points representing individual

TABLE 9
Ratio of blood volume to various body measurements

Patient	Blood volume	$\dfrac{\text{Blood volume}}{\text{Weight}}$	$\dfrac{(\text{Blood volume}) \times 10}{\text{Area (Du Bois)}}$	$\dfrac{(\text{Blood volume}) \times 10}{\text{Area (Meeh)}}$	$\dfrac{(\text{Blood volume}) \times 10}{\text{Area (Howland)}}$
	cc				
J G	220	100 9	1 264	1 099	1 233
L S	275	77 9	1 159	0 997	1 179
D M	290	81 7	1 280	1 047	1 186
F B	300	67 9	1 169	0 936	1 047
F T	320	69 9	1 143	0 975	1 087
M A.	320	66 5	1 032	0 944	1 048
J W	340	78 0	1 349	1 071	1 199
E E	345	93 2	1 431	1 216	1 376
L G	355	55 6	1 032	0 867	0 931
C. K.	370	112 1	1 713	1 403	1 592
L. H	385	74 0		1 079	1 188
S E	410	61 0	1 136	0 967	1 031
D B	440	64 1	1 306	1 024	1 086
R W	445	78 3	1 307	1 175	1 280
W P	455	66 0	1 218	1 055	1 120
J L	500	74 1	1 393	1 176	1 253
A, B	505	66 2	1 295	1 095	1 144
J P	535	84 3		1 311	1 409
M. K	550	96 0	1 725	1 444	1 571
A. H	560	73 9	1 412	1 220	1 275
M C.	565	68 4	1 430	1 161	1 196
M C.	600	72 3	1 515	1 230	1 266
A H.	620	85 0	1 590	1 386	1 459
J J	655	79 6	1 609	1 351	1 393
Number of Observations		24	22	24	24
Arithmetical Mean		77 0 ±2 6*	1 34 ±0 04	1 14 ±0 03	1 23 ±0 03
Standard Deviation		13 0 ±1 9	0 20 ±0 03	0 16 ±0 02	0 17 ±0 02
Coefficient of Variation		17±2	15±2	14±2	14±2

* The second figure gives the standard error

determinations will lie on both sides of a straight line the equation for which is obtained from the coefficient and is expressed by the for-

mula B V $= 61$ Wt $+80$ A simpler formula, and one which for this series gives an approximation almost equally satisfactory, is obtained from the average of the ratios of blood volume to weight, as expressed in table 9, and is represented by B V $= 77$ cc per kilogram

From a study of these values, it appears that body weight or surface area calculated by any one of the three formulas used gives a fairly close measure of blood volume even in the presence of such sharp deviations of these functions from the values expected on the basis of age as are offered in this group of patients   For these ratios we have tabulated the values in individual observations (table 9) and have calculated the average figures, standard deviations, and coefficients of variation, with their standard errors   Since the correlation of blood volume with surface area is not significantly closer than that of blood volume with body weight, there is no material advantage in stating the formula for obtaining the blood volume from surface measurement, since the latter is readily obtainable only by the use of other formulas such as those quoted, based on weight or weight-and-length measurements

While the multiplication of determinations may conceivably reduce these standard deviations and increase the accuracy of prediction of the figure for the total blood volume, it is equally likely that there will always remain a number of individuals who, by reason of a constitutional factor not sufficiently taken into account in formulas of this simplicity, will show values deviating more or less sharply from such limits   Our reasons for believing this to be true are based on the fact that we obtained duplicate determinations of blood volume in the case of two patients, L G and M K, whose points on the plotted graph of blood volume per kilogram were located at a greater distance from the line of the regression equation B V $= 61$ Wt $+80$—the one above, the other below it—than any other points in the scatter   For L G we obtained the value 355 cc on admission, 355 cc 11 days later, for M K, 485 cc on admission, 550 cc 17 days later   The first values in each case have not been included in the determinations subjected to analysis in this paper for the reason that at the time they were obtained both patients showed some clinical evidence of dehydration and it was thought that they might at the time be suffering a temporary disturbance in blood volume   However, while they are justly

excluded from the set of "normal values" they still serve effectively to corroborate the second set of figures and eliminate the likelihood of gross technical error in their determination

Our results differ, then, from those previously reported by other authors mainly in three ways our average values for blood volume per kilogram are considerably lower, the correlation between blood volume and various simple body measurements is closer than between plasma volume and those measurements, and finally, the scattering of our ratio values of blood volume per kilogram is considerably greater than that obtained particularly by Darrow, Soule, and Buckman (18) The first point of difference may be ascribed to our use of the carbon monoxide method as contrasted with their use of the dye method When the measurement of the circulating blood volume depends on the quantitative administration of a foreign substance the loss of which from circulation, whether it be by diffusion out of the vascular system or by dilution in reservoirs of plasma in the small vessels as claimed by Whipple (14), would result in a raising of the analytical figure, it is difficult to see how our results can be too low They may be too high, if muscle hemoglobin binds any considerable share of the respired carbon monoxide The second point of difference rests also on the choice of method, since the carbon monoxide method determines the blood volume directly, it is independent of additional error from hematocrit readings Finally, the wider scattering of our results may in all probability be ascribed to the variety of developmental and nutritional states represented in our series

SUMMARY

1 A technique is described for the determination in infants of the circulating blood volume by the carbon monoxide method, based on the successful use of this method in tests on adults

2 In comparison with the dye method, the carbon monoxide method gave results which were more uniform and showed a fair conformity with body weight

3 In a small series of determinations of blood volume in patients less than two years of age, the correlation of blood volume to body weight, to surface area, and to body length suggested a normal inter-relationship of these measurements

4   As an estimate of the circulating blood volume in infants, exclusive of the new-born group, the formula

Blood Volume in cc   =   (Body Weight in kgm) $\times$ (77 $\pm$13)

may be expected to give the correct figure in more than half the cases

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AN APPARATUS FOR THE PROLONGED ADMINISTRATION
OF ARTIFICIAL RESPIRATION

I A DESIGN FOR ADULTS AND CHILDREN

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INTRODUCTION

Up to the present time, artificial respiration has generally been administered either (*a*) by manual methods or (*b*) by forcing air under pressure into and out of the lungs by means of an insufflating apparatus

The first method was brought to its present state of development by Sir E A Sharpey Schäfer   The obvious defect in this method lies in the fact that the chest is never inflated, it is only deflated and then allowed to return to the normal position through its inherent elasticity This process is in the reverse direction to that which is normal and if, for any reason, the tone of the respiratory muscles becomes impaired, the chest will refuse to return to the normal position, thereby creating a situation in which adequate ventilation is rendered impossible

The second means of artificial respiration has reference to positive pressure devices such as the bellows (Keith, 1909) and to the lung motor and pulmotor, which force air in and out of the lungs, usually through a face mask fitted with inlet and outlet valves   Among rescue squads in this country and Canada, this general method has been discredited and discarded (Engineering Committee of the Conference on Electric Shock, 1928)

A wholly new method has recently been devised by Thunberg (1927)   The patient is placed in a chamber in which the pressures are alternately raised and lowered about 55 mm of mercury   Since the pressure changes are equal on both sides of the thoracic wall

there is no movement of the chest   The alternate rarefaction and compression of a fixed volume of air causes a volume flow which is proportional to the changes in pressure and which thus produces adequate gas exchange in the lungs

A fourth method was described by Doe (1889)   This apparatus was devised for resuscitating asphyxiated children   It consisted of a small box, over an opening in which a rubber dam was stretched The infant was so placed in the box that his nose and mouth were held against a hole in the rubber dam   Through a tube connected to the box a person outside alternately blew in and sucked out air   Although there is no reason to doubt the efficacy of such a method, the task of supplying the pressures in this way for long periods must have been arduous and the results rather uncertain

In South Africa, Steuart (1918) suggested the use of a mechanically operated device for inducing artificial respiration in children with anterior poliomyelitis   To quote the author   "The principle used is to place the child's thorax and abdomen in a rigid air-tight chamber communicating with a large bellows, which periodically causes a partial vacuum in the box "   Either positive or negative pressures, or both, were obtainable and could be regulated as to frequency and amount Apparently the author carried his work no further (so far as the literature indicates) than the suggestion of this device

Through newspaper reports, our attention was brought to a device patented (1928) by Eisenmenger of Vienna   We had an opportunity recently to make experiments with this apparatus on normal men and also to serve as subjects   A rigid leather dome-shaped piece lined with rubber is placed over the patient's chest, extended about over the region from the clavicle to the umbilicus, and strapped tightly in position   Alternate positive and negative pressures inside the dome are applied by an electrically driven diaphragm pump   Although the dome-shaped cover is not air-tight at any time, the pump produces any desired pressure up to about 400 cm of water, either positive or negative

The apparatus which we have developed (figs 1 and 2) is based on principles somewhat different from any of those mentioned   The patient's body is entirely enclosed in a cylindrical sheet-metal tank sealed at one end   At the other end is a flat lid, to which is attached

a rubber collar   The patient's head and part of his neck protrude through the collar, the head lying upon an adjustable support outside the tank   Since the patient is thus enclosed in an air tight chamber, the pressure around the body may be alternately raised and lowered while the head always remains at atmospheric pressure   When the
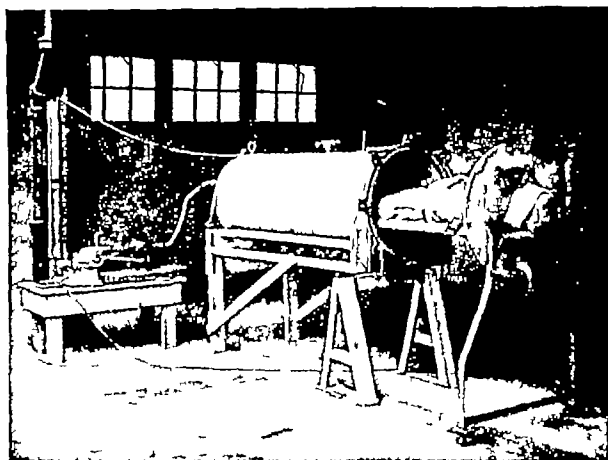
FIG 1 MECHANICAL RESPIRATOR, SHOWING TANK WITH PUMP MECHANISM CONNECTED BY FLEXIBLE TUBE AT FOOT END

The collar and head rest are in place and the patient is ready to be pushed into the tank.

pressure in the tank is lowered, the chest expands and air rushes into the lungs   When the pressure is raised, the chest is compressed and air is forced out of the lungs   By this method, movement of the chest is induced in such a manner as to simulate the natural respiratory movements

DESCRIPTION OF NEW MECHANICAL RESPIRATOR

The body of the respirator is made of metal (preferably sheet iron) welded at all joints   Rails in the form of "channels" are welded to the

inside   The patient lies on a mattress supported on an angle iron frame to which springs are hooked on all four sides   This bed frame is fastened rigidly to the lid of the tank, the bed and lid rolling in and out of the tank on wheels   The rubber collar through which the patient's head protrudes is fastened to the outside of the lid   The
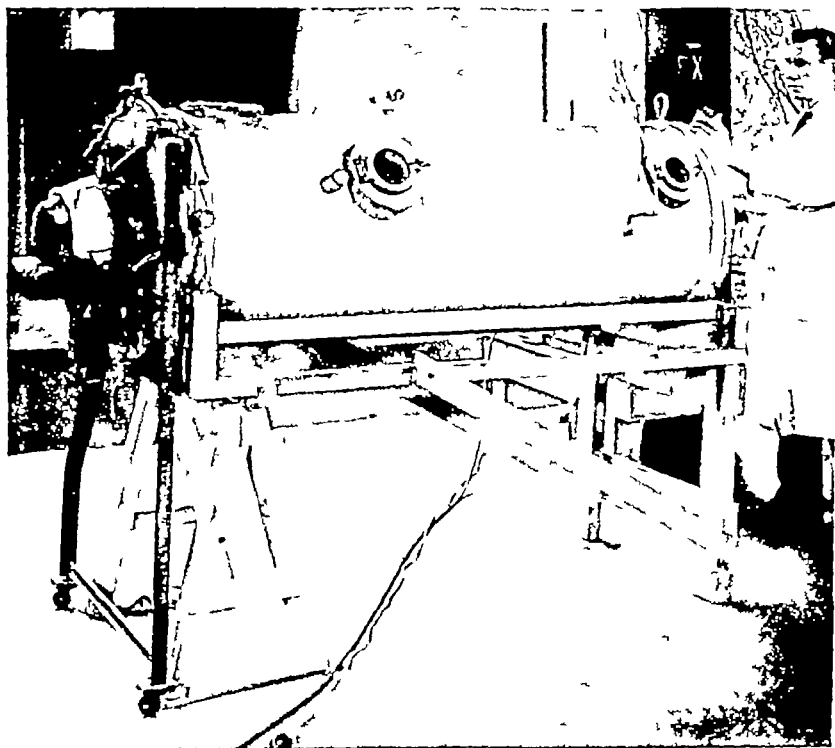
FIG 2 A PATIENT IN THE RESPIRATOR

Note the position of the port-holes   Attendant has his hands on the dial of the thermostat which controls the temperature and humidity of the air in the tank

patient can thus be taken out occasionally for examination without removal of this collar (fig 3)   To transfer a patient from a bed or stretcher to the tank requires about two minutes   If necessary, manual respiration can be carried on during this interval

The lid of the tank is clamped tightly against the body of the tank by means of refrigerator locks   On the inner surface of the lid there is a small ∧- shaped rim, which fits into a seamless rubber gasket dove-

tailed into the body of the tank   This arrangement assures a sufficiently tight seal on clamping the lid in place
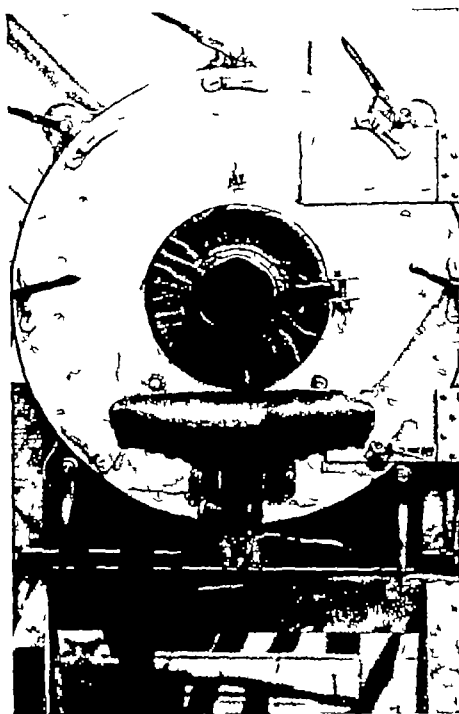
FIG 3 HEAD END OF TANK SHOWING ADJUSTABLE HEAD REST AND RUBBER
COLLAR

In future, the collars will be molded in a single piece and bolted to the lid of the tank by metal rings with wing nuts

The tank can be rotated about 75 degrees in both directions and the foot end can be raised or lowered about 15 degrees with the horizontal The chief purpose of these maneuvres is to give the patient a change in

position   If necessary, sides can be placed on the bed to keep the patient in position when the tank is rotated

The body of the tank is equipped with marine-type glass portholes for observing the patient and with numerous small holes (pipe taps) for inserting thermometers, attaching manometers, connecting pneumographs, blood pressure cuff, or stethescope   For taking x-ray photographs of chest movements, the glass of the porthole is rep'aced by a sheet of thin aluminium permeable to x-rays

The pumps (fig 1) which produce the pressure consist of two small universal blowers[1]   The valve mechanism controlling the pressure alternations is made up of two concentric cylinders, the outer one is stationary and the inner one driven by a worm gear connected through a variable speed motor   Holes are cut at certain intervals in both cylinders and these holes in turn lead out on one side to the pumps and on the other to the tank   The valves, driving gear, and cocks are so arranged that the pumps may be used in series, in parallel, or singly Either pump may be disconnected and replaced by a new one without stopping the apparatus   Any combination of pressures, positive or negative, up to about 60 cm of water can be applied and any number of pressure changes (i e , breaths) from 10 to 40 per minute are obtainable

The manometer which indicates pressures within the tank is filled with water colored red for visibility, the scale measures in half centimeters above and below the zero point (atmospheric pressure)   In controlling pressures, it is difficult to read both limbs of the manometer at the same time   We therefore use the rising limb only and, by measuring in half centimeters (thus doubling the actual reading) get the total excursion

The rubber collar (fig 3) was developed by the Hood Rubber Company   The diameter of the hole in the collar for the neck and of the hole in the lid of the tank through which the subject's head must pass and to which the collar is fixed were taken from figures supplied us by

[1] Manufactured by the Electric Blower Company, Boston, Mass   These blowers were chosen because they are designed for continuous service and deliver a considerable volume of air against 75 to 100 cm of water pressure   Although these pumps function satisfactorily, their noise is a drawback in hospital work We expect shortly to substitute pumps of more silent operation

the Cluett Peabody Company, collar manufacturers, and by the Knox Hat Company   Dimensions for the smaller collar sizes were taken from our own measurements on infants and children

Excessive movement of the rubber collar from the pressure changes is prevented by semi lunar discs of thin metal placed on both sides of the collar   This permits the use of thin rubber about the neck   At present, we are using a series of six collar sizes, which seems adequately to cover variations in neck sizes from children two years of age to adults

The rubber collars must withstand continuous pressure changes of from 0 to 60 cm of water without appreciable leakage, they must be actually air-tight at low pressures, and, at the same time, they must not be uncomfortable   Up to the present, all of our collars have been built by hand, but we hope soon to have them moulded in one piece, which would materially reduce their cost and increase their uniformity

PRESSURES REQUIRED TO INDUCE BREATHING

Our chief purpose in making this apparatus was to secure a device for treating patients suffering from protracted cases of respiratory failure, such as are induced by severe carbon monoxide poisoning, electric shock, morphine poisoning, poliomyelitis, and the like   The important question of how much pressure is needed to induce breathing in pathologic cases was estimated by using a number of normal men and women as subjects and observing their reactions when the pumps were working against various pressures

Table 1 gives the results of a series of experiments on normal men and women, chosen at random from the laboratory personnel in the building   The plot of these data (fig 4) shows that there is a very considerable variation in individual reactions and experiments like nos 11 and 12 indicate that the same subject does not necessarily react in the same way in two experiments under approximately the same pressures   As the normal minute volume itself varies as much as 10 or 20 per cent, it seems probable that the scattering of the points about the dotted line of figure 4 are within the normal expected variation

In order to determine approximate threshold pressures on a normal subject, one observes the manometer and gradually increases the

TABLE 1

Changes in minute volume resulting from various pressures

Experi ment	Subject	Vital capacity	Normal minute volume	Average plus and minus pressures applied	Resulting minute volume	Increase above normal	Approximate threshold pressure
		liters	*liters*	*cm water*	*liters*	*per cent*	*cm water*
1	A (m)	6 1	10	7 6	8 4*	−15 7	10
2				10 2	10 0*	0	
3				12 0	12 6*	25 7	
4				19 0	16 3*	63 0	
5				21 0	23 5*	135 0	
6				30 7	40 0*	300 0	
7	B (m)	5 8	5 8	4 3	5 5	−5 0	10
8				9 1	5 8	0	
9				11 6	7 7*	33 0	
10				14 0	10 8	86 2	
11				21 3	11 6*	100 0	
12				21 6	17 2	193 0	
13	C (m)	5 1	9	6 3	10 6	17 8	6
14				11 5	13 9	54 0	
15				19 1	19 0	111 0	
16	D (f)	3 2	5	7 9	4 4	−12 0	8
17				9 7	9 8*	96 0	
18				11 6	10 0	100 0	
19				17 1	14 8*	196 0	
20				21 5	22 2	340 0	
21	E (m)	4 5	8 2	8 2	15 4*	87 5	4
22				16 4	23 6*	188 0	
23	F (m)	5 6	8	5 0	11 6	45 0	2 noticeable
24				9 5	16 2	100 0	20 complete
25				15 1	19 6	145 0	control
26				20 3	34 7	334 0	
27	G (f)	2 4	6 6	6 8	12 7*	93 0	4
28				17 3	22 0*	233 0	

* Minute volumes determined in the course of taking Benedict metabolism records Other values determined spirometrically (Drinker, 1927)

pressures—positive and negative—by turning the proper valves When there is too little pressure to be effective, the subject's breathing is not in perfect synchronism with the alternations of the pump and the excursions of the water in the manometer are therefore very irregular
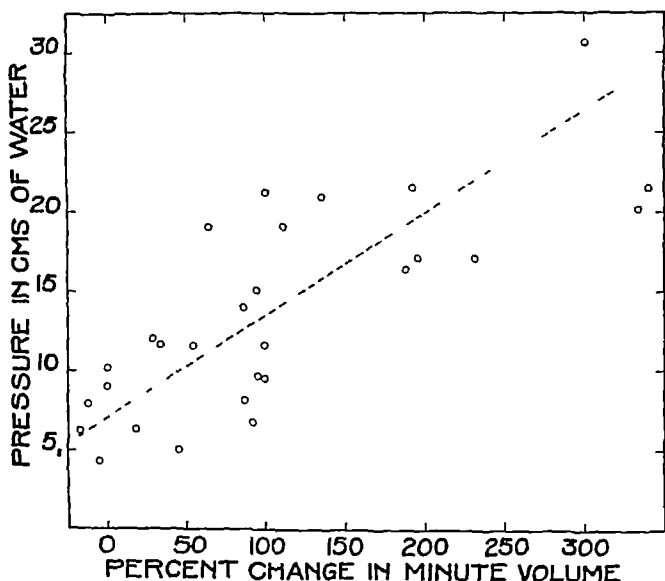
Fig 4 The Data of Table 1 Plotted as a Means of Determining Approximate Threshold Pressures—Pressures at Which the Respiratory Movements of the Average Subject Fall into Perfect Synchronism with the Pump

Where the dotted line passes through zero is the approximate threshold pressure

As the pressures are increased, a point is reached (generally at about 5 to 10 cm) when the subject falls into rhythm with the pump and remains so, no matter how much the pressure is increased subsequently For one man of normal physique (subject F, table 1) about

18 to 20 cm were required before the pump completely controlled his breathing   Another subject, A, a tall thin man, fell in at about 10 cm   With refractory subjects like F, synchronism with the pump is generally achieved by starting with pressures of about 18 to 20 cm and then rapidly coming down to 5 to 10 cm , which, from figure 4, seems to give about the average threshold range

The conditions encountered in the tank are quite unlike anything one experiences normally   The head is at atmospheric pressure and the chest, diaphragm, and abdomen are alternately under positive and negative pressures   Of the two, negative pressure (inducing inspiration) is much the more difficult to resist

There is no certain way of predicting the optimum combination for any individual   The most satisfactory procedure seems to consist in having all the controls as flexible as possible and quickly exploring the likely pressure ranges until a reasonable one is found to fit the patient

THE EFFECT OF ALTERNATE POSITIVE AND NEGATIVE PRESSURES UPON SPEECH

Another method of determining threshold pressures for normal subjects is given by the effect of pressure changes upon speech   A new subject—especially if he understands the apparatus—usually enters the tank with a certain degree of skepticism—he doubts the effectiveness of such small pressure changes   As the pressures are gradually increased, he is likely to attempt to talk or laugh and, as either function is cut off abruptly by inspiration, one can easily tell when the threshold pressure is approached   For the majority of subjects, speech is seriously hindered at pressures of about 5 to 10 cm and is almost wholly prevented at pressures of about 20 cm or more   This, of course, applies to the inspiration phase only

PRESSURES EXERTED IN ARTIFICIAL RESPIRATION BY THE SCHAFER METHOD

At our suggestion, Mr T J Shaughnessy, supervisor of resuscitation and instruction in the Consolidated Gas Company of New York, was so good as to have his rescue squads, numbering altogether about 45

men, perform the Schäfer prone pressure method on the platform of a spring scale which registered the pressure in pounds applied by the operator   All the subjects had repeatedly used the Schäfer method in actual resuscitation work in and about New York City   The results of the experiment, plotted in figure 5, show that the pressures applied ranged from about 41 to 63 kilos and varied roughly with the weight of the operator

FIG 5 A COMPARISON OF THE WEIGHTS AND PRESSURES APPLIED BY 45 EXPE RIENCED "PRONE PRESSURE RESUSCITATORS" FROM THE RESUSCITATION CREWS OF THE CONSOLIDATED GAS COMPANY OF NEW YORK
Courtesy of Mr T J Shaughnessy

This range is somewhat above the 23 to 41 kgm (50 to 90 pounds) given by Keith (1909) as practical working figures in applying the Shäfer method

Mr Shaughnessy's data are of interest to us because they show what relatively great pressures must be applied over a small area (the palms of two hands) in practical resuscitation work. In this case, of course, positive pressure only (forcing expiration) is exerted

THE EFFECT OF THE RESPIRATOR ON METABOLISM

If a subject is passive and readily falls into rhythm with the pump, it is reasonable to expect a consequent reduction in metabolism, for the normal muscular work of breathing is supposedly being performed in large part by the pump   A considerable series of careful experiments upon normal men and women did not, however, bear out this expectation   The results showed that, for the most part, metabolism was not affected significantly unless threshold pressures were considerably exceeded, then increases of 10 to 20 per cent were obtained, owing, apparently, to the muscular work of resisting over-ventilation

OVER-VENTILATION AND ITS SIGNIFICANCE

It is impossible to produce on a normal subject more than a slight degree of apnea by the Schafer method   This method also has little effect upon a subject who has previously breathed forcibly for some minutes and has already become apneic   The mechanical respirator, on the other hand, offers a convenient method for producing apnea, for forcing a resistant or apneic subject to breathe, and for studying recovery from apnea

Figure 6 shows a Benedict metabolism record of subject A in the tank   He first breathed normally, then with one pump running at 20 cm   pressure, then with two pumps at about 30 7 cm   The record shows the subsequent apnea which extended over a period of some four minutes   There was, clearly enough, an appreciable consumption of oxygen during these four minutes, although the respiratory movements were negligible

In making this particular record, the rubber flap valves ordinarily used in the Benedict apparatus to control inspiration and expiration were removed   Instead we used a small blower or "impeller" devised by Collins for use with the apparatus when the subject is breathing deeply, as in performing muscular work   In this way, oxygen at atmospheric pressure is brought continually to the subject, so that, as the oxygen in the lungs is converted into carbon dioxide, more oxygen is drawn in to replace it   This process can take place with no visible chest movement

It may well be objected that the data obtained upon healthy sub-
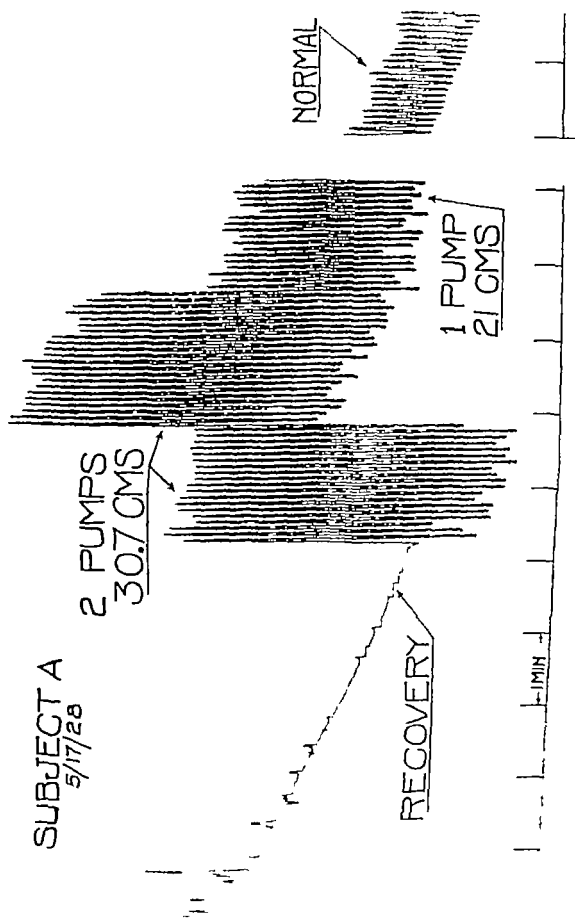
FIG 6 THE EFFECT ON A NORMAL MAN OF THE MECHANICAL RESPIRATOR, AS SHOWN BY THE BENEDICT PORTABLE METABOLISM APPARATUS

Record reads from right to left Abscissae represent time and ordinates oxygen consumption Note the pronounced oxygen consumption in the recovery (repnet) after the pumps had been shut off

SUBJECT A
5/17/28

2 PUMPS 30.7 CMS

1 PUMP 21 CMS

NORMAL

RECOVERY

1MIN

jects are not valid and may only reflect the fact that our subjects (table 1) find it easier to cooperate with the respirator than to assume a passive attitude   Such an objection, however, may safely be disposed of by considering the ease with which the subject can be over-ventilated to any desired degree (fig 6)   It has been demonstrated by
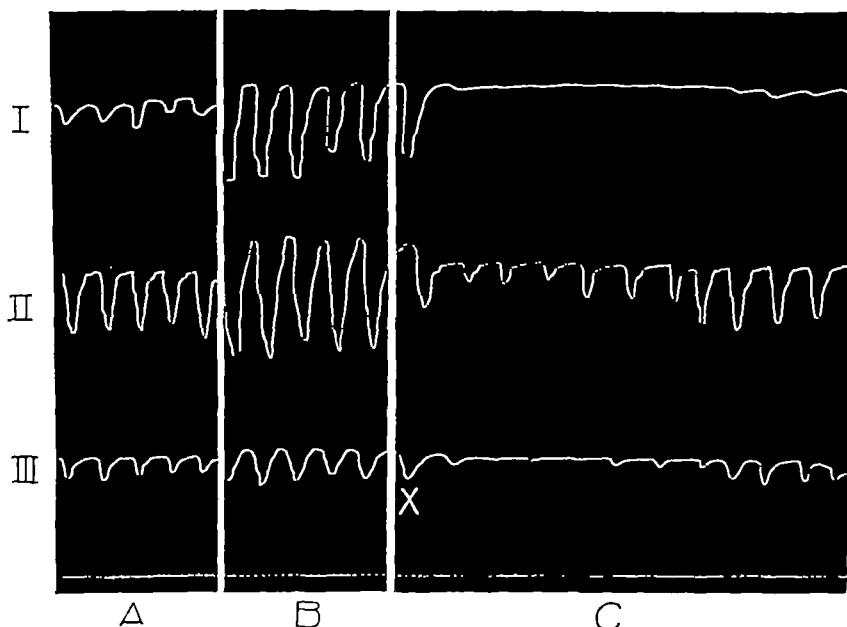
FIG 7 PNEUMOGRAPHIC RECORDS TAKEN ON NORMAL SUBJECT

I, Porter pneumograph is placed around chest at level of the fourth rib, II, pneumograph is 2 5 cm below the tip of the xyphoid, and III, pneumograph is 13 cm below position in II   *A* represents normal breathing, *B* forced breathing with the pump delivering an average of + 12 and −10 cm of water pressure, and *C* recovery after the pump had been shut off at point *X*   Time intervals = 1 second

laboratory experiments upon mammals that over-ventilation is resisted by a muscular effort no less pronounced than that which follows under-ventilation

In order to prevent over-ventilation and the consequent development of alkalemia in the case of a patient suffering from respiratory failure, it is best to collect the expired air from time to time in a spirometer and determine the minute volume   The latter should then be

regulated to conform with the average respiratory volume per minute per kilogram of body weight for the particular individual

CHEST AND DIAPHRAGMATIC MOVEMENTS

Pneumograph records

In order to study the relative movement of the chest and diaphragm in natural and in mechanically forced breathing, three Porter pneumographs were placed on subject A, connected so as to write separate records on a kymograph equipped with a second marker

The first pneumograph, I, was placed at the level of the fourth rib, the second, II, 2 5 cm below the tip of the xyphoid, and the third, III, 13 cm below the second   A record of normal breathing was then made (fig 7, A) with the subject lying at rest in the tank   Next the pump was run at pressures of +12 and −10 cm giving the record shown in figure 7, B   Finally, the pump was stopped and the period of recovery from the slight apnea produced is shown in figure 7, C

Compared with normal breathing, breathing induced by the mechanical respirator showed a marked increase in chest movement, particularly in movement of the upper part of the chest   Directly after the pump was stopped, there appeared to be practically no movement in the first and third pneumographs and a slight movement in the second As recovery progressed, diaphragmatic breathing (the third pneumograph) became more pronounced and, after about one minute, was practically normal   The upper pneumograph shows that normal breathing had not been resumed about one minute after the pump was stopped

X ray record

A portable x ray machine was arranged so that the tube was directly over the central porthole, near the head end of the tank (fig 2)   By replacing the glass of the port with thin aluminium, x-ray photographs of the subject's chest movements could be taken   The subject in this case was an athletic man 27 years of age, apparently in excellent health His vital capacity was 4 5 liters

Dr Merrill C Sosman[2] was kind enough to take the pictures for us and commented upon the resulting plates as follows

[2] Roentgenologist in-chief Peter Bent Brigham Hospital Boston

Films of the chest on deep voluntary inspiration and expiration show a maximum movement of the diaphragms 8 8 cm , with a change in diameter of the chest of 3 cm

Films of the chest in the respirator, one at 46 cm of water, negative pressure (inspiration), and the other at 39 cm of water, positive pressure (expiration) show a change of 10 5 cm in the height of the diaphragms and a much more marked change in the width of the chest   The latter cannot be measured, as at expiration the edges of the chest are beyond the limits of our largest film   It is estimated to be about 6 cm   A comparison of the two sets of films shows that forced inspiration and expiration cause more change in aeration of the lungs than do voluntary inspiration and expiration

THE APPARATUS USED AS A MEANS OF RECORDING RESPIRATORY MOVEMENTS

When the subject lies at rest in the respirator breathing naturally without the mechanical assistance of the pump, the expansion of the chest caused by inspiration raises the pressure of the air in the respirator and, conversely, the deflation of the chest caused by expiration lowers the pressure   If now a manometer of high sensitivity be connected to the respirator, the respiratory movements can be easily followed   The sensitivity of such a manometer may be greatly augmented by tilting it at an angle of 5 or 6 degrees with the horizontal and by using a liquid of low specific gravity, such as ether

By treating our respirator as a body plethysmograph, the respiratory movements may be recorded volumetrically   This may be effected by connecting the respirator with a Krogh spirometer of 5 or 6 liters capacity, with a writing point in apposition with a kymograph At inspiration, a volume of air equivalent to that inspired is displaced into the spirometer by the expansion of the chest and at expiration the air is again withdrawn from the spirometer by the contraction of the chest   The alternate movements of the spirometer lid writes a record both of respiratory movements and of respiratory volumes The accuracy of the latter is directly proportional to the sensitivity of the spirometer and inversely proportional to the volume of air surrounding the body, since a certain slight pressure must be built up in the plethysmograph to overcome the inertia of the spirometer

This method of recording respiration has been previously used by Shaw (1928) and also by Binger and Davis (1928)

Since the tank can be changed at will from a mechanical respirator to a plethysmograph, it gives a convenient and clinically practical method of observing in pathologic cases whether the subject's normal breathing is being resumed

CONTROL OF TEMPERATURE AND HUMIDITY

A thermostat for controlling the temperature and humidity of the air in the tank is screwed in at the foot end near the top   When the tank is completely shut—when the pumps are not running and no air is being introduced from outside—the patient's body heat raises the temperature within to an uncomfortable degree, if some sort of thermostatic mechanism is not provided   In our apparatus, the air within the tank is circulated through a can of ice, which serves both as a dehumidifier and a cooling agent   The small blower, producing the air circulation, is controlled by this thermostat

When the pumps are running, the air is continually being changed—the air withdrawn from the tank is exhausted and blown into the room, without finding its way directly into the tank again   Then thermostatic control is not needed

DISCUSSION

We recently had an opportunity to apply our respirator (1929) in a case of intercostal paralysis from poliomyelitis   The patient, a child of eight years, was in the machine almost continuously for a period of 122 hours, at the end of which time death occurred, resulting apparently from cardiac failure brought on by an extensive bronchopneumonia in the right lung   Examination of the lungs at autopsy showed no evidence of trauma from overinflation, nor was any other form of damage to the patient from this prolonged application of artificial respiration observed   During the time the child was in the respirator, she was able to talk, sleep, and take nourishment while the pumps were running

This case and another described by Petrén and Sjövall (1926) indicate that respiratory failure in cases of poliomyelitis can be prevented and encourage the hope that recovery may follow in certain cases which would otherwise terminate fatally

Our respirator in its present form, like Thunberg's Barospirator, is a rather cumbersome and complicated apparatus—it is a clinical tool to be used in hospitals under the direction of competent clinicians For emergency rescue work, it cannot displace the Schafer method of manual resuscitation

SUMMARY

The literature on mechanically operated devices for the application of artificial respiration is briefly reviewed and a new type of respirator is described and illustrated   This respirator consists in a metal tank, sealed at one end, in which the patient is placed, his head protruding through a rubber collar attached to the open end of the tank   By electrically operated pumps, the atmosphere within the tank is placed alternately under positive and negative pressure, positive pressure inducing expiration and negative pressure inspiration   The pressure changes per minute can be varied from 10 to 40 and any combination of pressures, positive or negative, from approximately 60 cm of water to atmospheric pressure can be obtained   The average threshold pressures—those which induce breathing against the subject's will— are found to be about 5 to 10 cm of water for normal men and woman Apnea can be produced by means of the respirator and an apneic subject can be made to breathe deeply

By turning a valve at the foot end, the respiration tank is converted instantly into a plethysmograph   If a suitable spirometer is attached, graphic records of breathing can be made and the patient's ability to breathe without assistance determined

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THE INVOLUTION OF CUTANEOUS XANTHOMATA CAUSED BY DIETS LOW IN CALORIES

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Since the demonstration of cholesterol crystals in xanthomata by Bazin (1) in 1869 and the subsequent finding of a hypercholesterolemia by Chauffard and Laroche (2), cutaneous xanthomata have been considered cholesterol tumors. It has been pointed out by Wile, Eckstein, and Curtis (3) that xanthomatous tumors do not have as much cholesterol in them as does the surrounding skin and that their presence does not necessarily depend upon a lipemia or a hypercholesterolemia. In this same study it was demonstrated that not more than 11 per cent of the tumors was neutral fat and 16 per cent of this was cholesterol. Major (4) has shown that xanthomatous nodules consist of neutral fat, fatty acids, lipoids, and cholesterol. It is a common observation that xanthomata associated with hyperglycemia are often seen in obese individuals and as these tumors consist of neutral fat, fatty acids, lipoids, and cholesterol they may represent deposits of fat in the skin serving the same purpose as other fat depots in the body. If such a premise be true these cutaneous deposits of fat should respond to dietary measures as other fat depots do. Such was shown in our first paper (3) to be true, at least in part. In order to investigate this hypothesis further we have studied three patients with xanthomata. The first two patients were sisters who developed xanthomatous lesions within six months of each other without an associated diabetes. The third patient had a severe form of cutaneous xanthomata and diabetes mellitus. All patients had a lipemia.

CASE HISTORIES

Case 1 K. L., female, age 8, came to the hospital complaining of an eruption which began 1½ years ago as a glass pin head size yellowish hypertrophic growth on
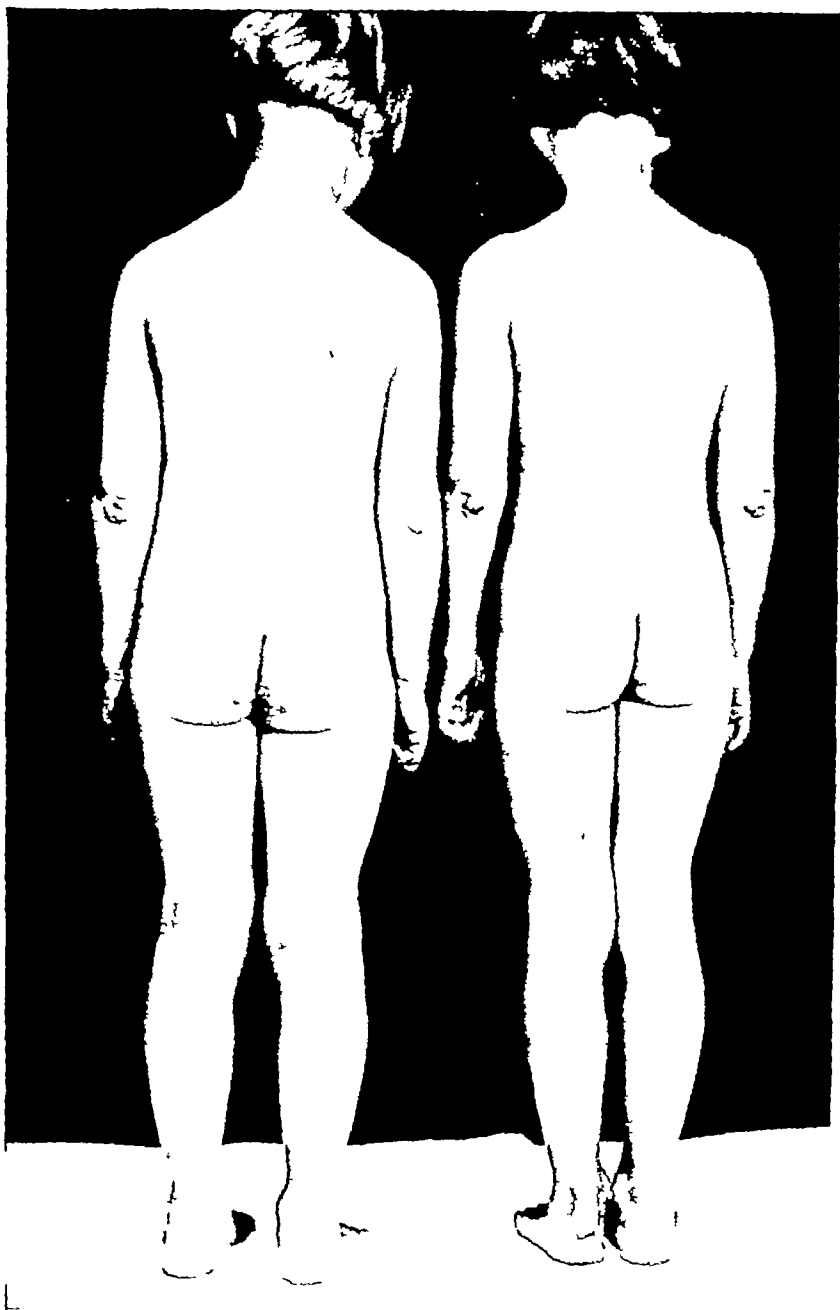
FIG 1   CASE 1, K L , AND CASE 2, M L , SHOWING THE DISTRIBUTION OF THE
XANTHOMATOUS LESIONS AND THEIR NODULAR CHARACTER

the dorsal surface of both elbows   The nodules were yellow in color and painless
They slowly increased in size and in number until they involved all extremities and
the trunk   Except for measles, mumps, whooping cough, and chickenpox, her
past history is essentially negative   Neither has she had any symptoms referable to
diabetes mellitus nor has there been any family history of the disease   On the
anterior aspect of the body she presented three small lesions on the outer aspect
of the right knee and two small lesions on the upper outer aspect of the left knee
These nodules varied approximately from 1 to 10 mm in diameter   They
were sharply elevated from the surrounding skin, orange yellow in color, and
firm to palpation   On the posterior aspect of the body the lesions were located
over the elbows, in the gluteal cleft and over both tendo Achilles   The lesions
for the most part were made up of groups of nodules varying in diameter from 1
to 15 mm   They were orange yellow in color, sharply elevated from the sur
rounding skin, and firm to palpation   (See fig 1)   Clinical diagnosis   xan
thoma tuberosum   Pathological diagnosis   xanthoma tuberosum

Case 2   M L , age 7, a sister of K L came to the clinic complaining of a small
nodular eruption which had begun two years ago on the dorsal aspect of both
elbows   The lesions slowly increased in size and in distribution until they were
present over all extremities and the trunk   Her past history is negative except
for measles mumps, chickenpox and whooping cough   No symptoms of diabetes
mellitus were present
   On the anterior aspect of the body she presented two small lesions on either side
of the glabella which were approximately 1 to 10 mm in diameter   On the volar
surface of the right wrist were two lesions one approximately 15 mm and one
approximately 2 mm in diameter   Over the outer aspect of both knees there
were similar lesions slightly larger than those of the arms   Most of the lesions
were orange yellow in color, sharply raised from the surrounding skin and firm to
palpation   Over the posterior surface there were lesions on the neck which were
verrucous and linear appearing something like a naevus   Over the elbows and in
the popliteal space the lesions were made up of a confluence of smaller lesions
varying in size from approximately 10 to 50 mm in diameter   Over the lower
aspects of the tendo Achilles the lesions were somewhat smaller   (See fig 1)
Clinical diagnosis   xanthoma tuberosum   Pathological diagnosis   xanthoma
tuberosum

Case 3   B B , male, age 42   came to the hospital because of an extensive
eruption all over his body   Five years previously he noticed a small papular and
nodular eruption on his shoulders and the extensor surfaces of his arms   The
lesions were yellow, moderately firm and discrete   They rapidly spread until
they involved the whole body with predilection for the buttocks, extensor surfaces
of the arms and legs   Since then the lesions have persisted and are painful when
traumatized

The patient was an obese male (190 pounds) who presented over the trunk, elbows, knees, buttocks, and backs of the hands large numbers of typical tuberous xanthomatous lesions   The older lesions were quite hard and tender on pressure   The color of the lesions was a chamois yellow   The urine showed a 4+ sugar and the blood a definite hyperglycemia and lipemia

Diagnosis diabetes mellitus, obesity xanthoma tuberosum

METHODS

Case 1, K L , and case 2, M L , were hospitalized July 2, 1928, and placed on a diet consisting of 70 grams of protein, 150 grams of fat, and 100 grams of carbohydrate   Two basal metabolism determinations were done on each patient on successive days   The 24 hour basal calories were then calculated for both case 1, K L , and case 2, M L , from Sanford's Tables for their height, weight, age sex, and basal metabolism   These determinations were followed by a glucose tolerance test which was repeated at the conclusion of the low calorie diet periods   Daily weights were taken on each patient before breakfast clothed only in the standard hospital night-grown   Specimens for blood lipoid determinations were drawn at the same time the first glucose tolerance test was done, 10 days later, and 30 days later   Routine daily urines were done   Case 3, B B , was placed on four different types of diets   The first was the usual series of diets used by this hospital to determine the tolerance for glucose in diabetic patients, the second a definite reduction diet, the third a high fat and high calorie diet, and the fourth a maintenance diet   Glucose tolerance tests were done at the beginning of the experiment and at its end   Daily urines were examined during all periods except the second   Only one urine analysis was obtained at the end of this period   Changes in his weight were recorded at the end of each dietary period

Four blood lipoid determinations were made, two on separate uncontrolled diets, one while on the high calorie diet, and one while on the maintenance diet

DATA

It will be seen from table 1, that case 1, K L and case 2, M L , were placed on diets of 2030 calories   The 24-hour basal calories of case 1, K L , were determined on successive days to be 1050 and 1090 calories   The 24-hour basal calories of case 2, M L , were determined

on successive days to be 980 and 960 calories   It is evident then that this diet was well above their maintenance calories   They remained on this diet for 6 days   A slight increase in the firmness of the tumors and the depth of their color was noted   No marked change in weight occurred   For 8 days following this they were placed on diets of 50 grams of protein, 80 grams of fat, and 50 grams of carbohy-

TABLE 1

The effect of various diets on xanthomatous lesions   Case 1 K L , and Case 2 M L

Date	Diet				Weight		Blood fat		Effect on tumors
	Protein	Fat	Carbohydrates	Calories	Case 1 K L	Case 2 M L	Case 1 K L	Case 2 M L L	
	gm	gm	gm		lbs	lbs	mgm per 100 cc	mgm per 100 cc	
July 3–9	70	150	100*	2 030	53½ 53½	52 52	1 383	1 330	Increased in hardness. Color deepened to orange yellow
July 10–17	50	80	50*	1,172	53½ 53½	52 52½	1,340	1,350	No change
July 18–26	40	20	40*	500	53½ 52½	51½ 51½			No change
July 27–August 6	35	13	39	413	52½ 48½	51½ 48½	1 183	1,030	Beginning involution
August 7–10	House diet			?2,500–3 000	48½ 55	48 52½			Increase in size firmness and depth of color of all tumors

* Plus an unknown number of calories obtained surreptitiously from other patients.

drate with a total of 1120 calories   No effect was observed on the tumors and no change occurred in the patients' weight   We felt that this diet was too near their maintenance calories, even though they were up and about the ward, so they were placed on diets of 40 grams of protein, 20 grams of fat, and 40 grams of carbohydrate having a total of 500 calories   Two hours of exercise in the gymnasium was

done daily One day after the diet was begun it was found that both patients had been given peanuts by their family and that during their wanderings about the ward, patients had given them candy, fruit, and food from the hospital trays The patients hence were strictly con-
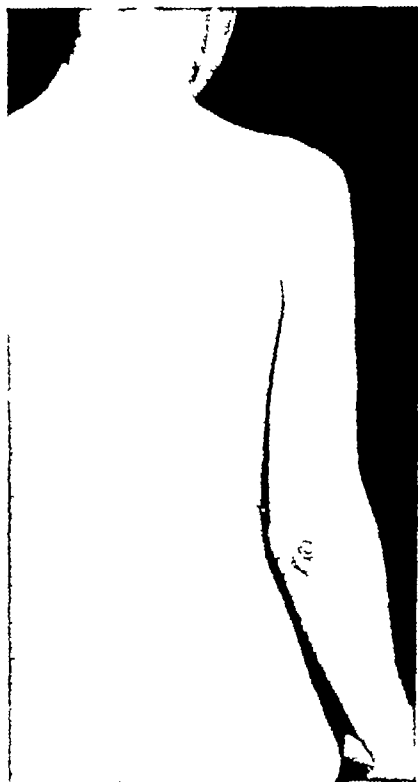
FIG 2, A   FIG 2, B

FIG 2 (4) CASE 1, K L, SHOWING THE SIZE AND NODULAR CHARACTER OF THE XANTHOMATOUS LESION ON THE RIGHT ELBOW BEFORE TREATMENT (B) CASE 1, K L, SHOWING THE DECREASE IN SIZE AND FLATTENING OF THE SURFACE OF THE LESION AFTER A SHORT PERIOD OF TREATMENT

fined to their rooms and for the first time a definite loss of weight occurred Nine days later their diets were further reduced to 35 grams of protein, 13 grams of fat, and 39 grams of carbohydrate, having a total of 413 calories A rapid loss of weight occurred With the beginning of the weight loss there was also noted a definite softening of the

tumors   The orange yellow color began to fade and a pink hue developed   (See fig 2 and 3)   It will also be seen from table 1 that the blood lipoids which had formerly been high, now showed a definite decrease

Unfortunately at this point we were notified that the children could only remain in the hospital 5 days longer so they were immediately put on house diets   Their weight increased rapidly to a level greater
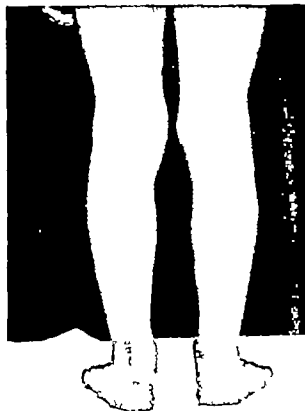
FIG 3, 1   FIG 3, B

FIG 3 CASE 1, K L, (4) BEFORE TREATMENT AND (B) AFTER TREATMENT
Note the decrease in the size and fullness of the nodular eruption on the margins of the right and left popliteal spaces and the left heel

than at their entrance   The lesions again became firm and the orange yellow hue returned

The urine examinations throughout the experiment were negative except for numerous fat droplets which disappeared while on the last low calorie diet   The glucose tolerance curves (see fig 4) from these patients suggest an inability to tolerate large amounts of glucose

It will be seen from table 2 that case 3, B B , a mild diabetic, when placed on a series of desugarization diets in which the fat was constant and the calories were increased by the addition of glucose, experienced

an involution of his tumors and a loss of weight   This dietary pro-
cedure was followed by a diet consisting of 50 grams of protein, 50
grams of fat, and 200 grams of carbohydrate   While on this diet he
lost 17 pounds in 30 days and his tumors continued to involute   He
was then placed on a diet high in calories and fat for 30 days   This
diet consisted of 55 grams of protein, 210 grams of fat, and 300 grams
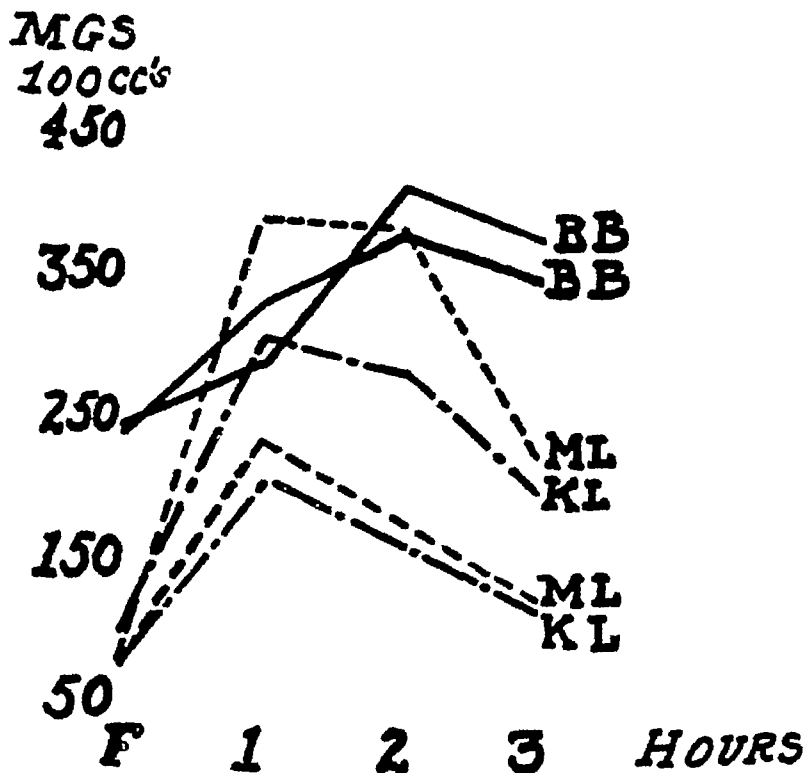
FIG 4 THE TYPICAL DIABETIC INTOLERANCE FOR GLUCOSE OF CASE 3, B B
AND THE ATYPICAL CURVES OF CASES 1, K L , AND 2, M L , ARE
SHOWN IN THIS FIGURE

of carbohydrate with a total of 3310 calories   During this period he
gained 8 pounds weight and had a reappearance of all his tumors   He
also had a glycosuria and acetonuria   Following this he was given a
maintainence diet consisting of 55 grams of protein, 210 grams of fat
and 100 grams of carbohydrate with a total of 2510 calories   It will
be noted that the fat of this diet is the same as the former diet but the

TABLE 2

Effect of diets high in calories and low in calories on xanthomatous lesions   Case 3, B B

Diets				Dura tion of diet	Weight gain or loss	Blood fat	Effect on tumors
Protein	Fat	Carbo-hydrates	Calo-ries				
grams	*grams*	*grams*		*days*		*mgm per 100 cc*	
House diet			?		Gain	2 275	
55	210	35	2 240	3		2 215	Beginning involution
55	210	50	2,300	3			Beginning involution
55	210	100	2 500	3	Loss of 12 pounds		Beginning involution
55	210	150	2,700	3			Beginning involution
55	210	175	2,800	3			Beginning involution
50	50	200	1 450	30	Loss of 17 pounds		Marked involution
55	210	300	3 310	30	Gain of 8 pounds	2,416	Reappearance
55	210	100	2,510	45	Little change in weight	1,175	Complete involution
55	210	100	2 510	180	Little change in weight		Complete involution, all tumors replaced by scar tissue
Unmeasured diet			?	30	Gained 5 pounds	2,225	Reappearance of three tumors

TABLE 3

Influence of fat on blood lipoids

	Blood fat	Increase
Patient B B case 3		
	mgm per 100 cc	*per cent*
Fasting	2 300	
2 hours after 100 cc. of olive oil	2 630	14
4 hours after 100 cc. of olive oil	2 769	16
6 hours after 100 cc. of olive oil	2 337	2
Patient S, normal blood fat		
Fasting	439	
2 hours after 100 cc. of olive oil	467	6
4 hours after 100 of olive oil	600	37
6 hours after 100 f olive oil	508	13
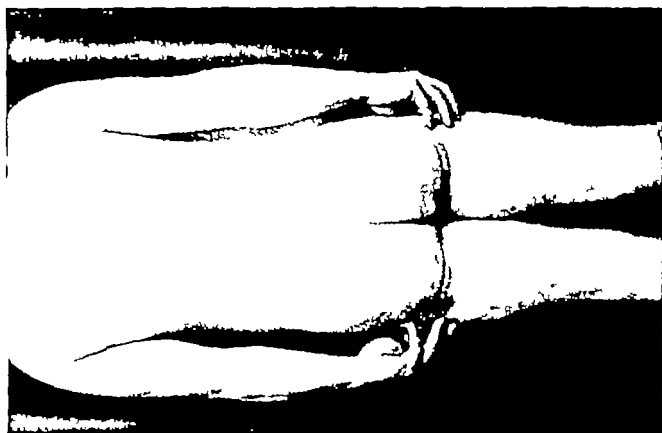
Fig 5, C
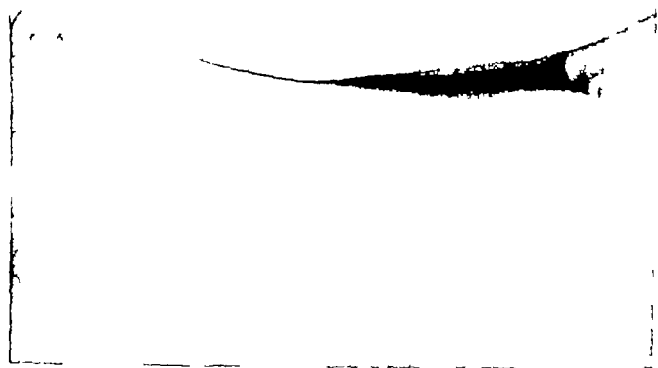
Fig 5, B

THE SURFACE TENSION OF BLOOD SERUM, AND THE DETERMINATION OF THE SURFACE TENSION OF BIOLOGICAL FLUIDS

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INTRODUCTION

This investigation was undertaken in order to obtain a more exact knowledge of the surface tension of undiluted blood serum, and its variation under various clinical conditions, than is given by the work reported earlier by others (1–12)   It was found that practically all previous investigators have made errors in the experimental procedure or in the calculation of their results, usually in both, of such a high magnitude as to make their data almost entirely worthless   Thus a new field is opened up for future investigation

The mean value obtained for the surface tension of undiluted normal blood serum is 52 dynes per centimeter at 20° and 48 dynes at 37°C The result at the higher temperature agrees moderately well with the mean value found by Morgan and Woodward (7), but it is known that the method by which their results were calculated gives values which are too low   The average of all of their values at 37° is 45 5 while the average of all of our results is 46 2   However, their work represents a great forward step, as it is of a much higher degree of accuracy than that of any of the other earlier investigators   Almost all of the values obtained by others are of an altogether different order of magnitude, since they are from 10 to 20 dynes too high

The worthlessness of much of the work is due to an altogether incorrect assumption, which is that if only relative values are desired no attention needs to be paid to the theory of the method used   Thus the method in which the number of drops given by a certain volume of the liquid with a certain tip (drop number method) is supposed to be inversely proportional to the surface tension, is practically useless,

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as is the ring method as commonly used   The determination of capillary height is the most accurate of all known methods for determination of the surface tension of pure liquids which are not viscous   When used with blood serum, and with most other biolog liquids, it has, however, the disadvantage that the film at the surf often prevents the attainment of capillary-hydrostatic equilibri and it may affect the angle of contact   The bubble pressure met suffers from the disadvantage that as usually applied the experimer procedure does not agree with the conditions set up by the theory the method   A much greater disadvantage is that it is difficult almost impossible to hold the bubble at its maximum pressure the considerable time necessary to establish equilibrium between interior of the serum and its surface

A careful consideration of all of the available methods seemed indicate that the best results for such liquids may be obtained the special modification of the drop weight method described later the paper   The drop weight method avoids difficulties which rel to the angle of contact and the measurements are much less affec by the surface viscosity than when the capillary height method is us

THE DROP WEIGHT APPARATUS FOR THE DETERMINATION OF SURFA
TENSION OF BLOOD SERUM AND OTHER BIOLOGICAL LIQUIDS

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The apparatus (fig 1) consists essentially of an inverted capilla U-tube, suspended in a metal box (B), which is provided with gla windows in front of and back of the tip from which the drop of sert is suspended   One limb of this inverted U-tube dips into a reserve (S) of the serum   The other limb ends in the tip which is held by stopper of glass or metal in the weighing bottle (V), in which the dro are collected as they fall

Each drop is pulled over, until it attains the maximum size consis ent with stability, by the action of suction applied through the met tube (A), and the drop is held at this maximum extension for seve minutes before it is allowed to fall

Fig 1 Apparatus for the Determination of the Surface Tension of Blood Serum and Other Biological Liquids by the Drop Weight Method

V, weighing bottle under tip for collecting drops, S, reservoir for serum, KCR, rod for controlling the speed of drop formation, P, rack and pinion for adjusting height of rod R, F, tube for saturating serum with alveolar air, A, suction tube, and B, box for keeping out water of thermostat. The tip of pyrex glass is circular and shows no flaws in its edge under a magnification of $20\times$ The bottom of the tip is first ground off plane   Then a circular piece of glass of the same diameter as the tip is sealed over it by means of de Khotinsky cement. The sides of the tip are then ground down to the required diameter in a precision lathe   The tip is rotated in one direction, and it is ground by a high speed, very fine, disc of car borundum, which rotates in the opposite direction and is finally given a high polish by means of rouge.   When the cement is melted a tip ground properly in this way is found to be circular and perfectly sharp   Eight different diameters of the tip are measured to 0 0001 cm each

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V, weighing bottle under tip for collecting drops, S, reservoir for serum, KCR, rod for controlling the speed of drop formation, P, rack and pinion for adjusting height of rod R, F, tube for saturating serum with alveolar air, A, suction tube, and B, box for keeping out water of thermostat.   The tip of pyrex glass is circular and shows no flaws in its edge under a magnification of $20 \times$ The bottom of the tip is first ground off plane   Then a circular piece of glass of the same diameter as the tip is sealed over it by means of de Khotinsky cement.   The sides of the tip are then ground down to the required diameter in a precision lathe   The tip is rotated in one direction, and it is ground by a high speed, very fine, disc of carborundum, which rotates in the opposite direction and is finally given a high polish by means of rouge   When the cement is melted a tip ground properly in this way is found to be circular and perfectly sharp   Eight different diameters of the tip are measured to 0 0001 cm each

The glass tube (F) is used for the aeration of the serum with alveol
air, as described in the section on aeration, or with another gas

During a determination the metal box (B) is kept entirely immers
in the water of a thermostat at the proper temperature $\pm0\ 01°$

It is extremely important that the serum in (S) be adjusted to t
level requisite to hold the drop at exactly full extension for the sev
minute period   This is done by means of the rod (R), which contr
the level of the reservoir (S) For the work described in the prese
paper this level was adjusted by hand, and the delicate control
the drop was effected by properly varying the suction applied at $(A$

However, the difficulty of controlling the drop in this way led to t
method of control described in the next paragraph

CONTROL OF THE DROP AND VARIATION OF THE DROP WEIGHT WITH TI

Biological liquids contain in general surface-active substances, a
the surface whose tension is being measured must be kept fully form
sufficiently long to allow equilibrium between the surface and t
interior of the liquid to be attained   With aqueous solutions
decylic acid the minimum period of full extension is more than thir
minutes   With blood serum the surface tension falls for sever
minutes, and rises again if the surface becomes older than about sev
minutes   This actual or apparent increase may be due to the form
tion of a solid film which does not represent a true condition of equi
brium with the interior of the liquid

A delicate adjustment of the pressure in the drop, which determin
its extension, is obtained by suspending the rod (R) from the bar $($
which is raised and lowered by a rachet and pinion   For this purpo
a microscope stand with a coarse and fine adjustment is ideal

The "full extension of the drop" can be determined only by tri
The telescope of a cathetometer is levelled and the height of t
telescope is so adjusted that the horizontal cross hair and the bottom
the drop at full extension appear coincident   The level of the seru
in (S) is so adjusted that the bottom of the drop will remain at th
level for the desired period (of seven minutes)   Several trials a
necessary, even with the experienced worker   A common error is
consider that the level of full extension lies higher than is actual
the case   To avoid this the level of the cross hair is lowered slightl

and an endeavor is made to adjust the level of the bottle (S) in such a way that the drop will not fall   If this is possible, the preceding level was too high

An error in the opposite direction is apt to arise due to the decrease of the surface tension as the surface active substance diffuses into the surface from the body of the liquid   The length of the drop at "full extension" for the higher surface tension is greater than that for the lowest, or equilibrium tension   This makes it necessary, if a seven minute period is to be allowed, to determine the full extension at the end of this period

TABLE 1

Comparison of the weights of drop held at almost full extension for seven minutes and those held for a shorter period

Patient	Time	Drop weight	Time	Drop weight
	minutes		*minutes*	
D	$\frac{1}{2}$	0 06260	7	0 05393
F	$1\frac{1}{4}$	0 06070	7	0 05520
Γ			7	0 05440
S	1	0 05510	7	0 04770
S			7	0 04780
S	1	0 05860	7	0 04932

The tip used in the work in this and later tables on patients D to K inclusive has a diameter of 0 5944 cm   The tip used on all other patients has a diameter of 0.5000 cm

It should be kept in mind that what is called full extension of the drop does not correspond to the greatest length which the drop may have, but to the greatest length which it may have without becoming unstable   Almost immediately after the drop becomes unstable, the neck of the drop narrows with great rapidity, that is, the drop pinches off by a lateral movement of its upper surface (fig 2)

It was found that the use of a period of about seven minutes gave the minimum drop weight   If held longer than seven minutes, the drop weight seems to rise again, but this rise is probably due to the formation of a solid film   Thus, a sample which gave a drop weight of 0 05742 for a set of drops each held for a period of from 4 to 8 minutes, gave a drop weight of 0 05790 for a drop held twenty-one minutes   Table 1 shows a comparison between drops held seven
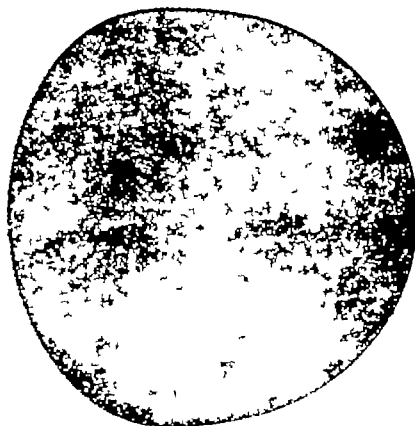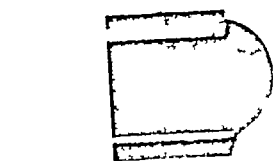
Fig 2, C
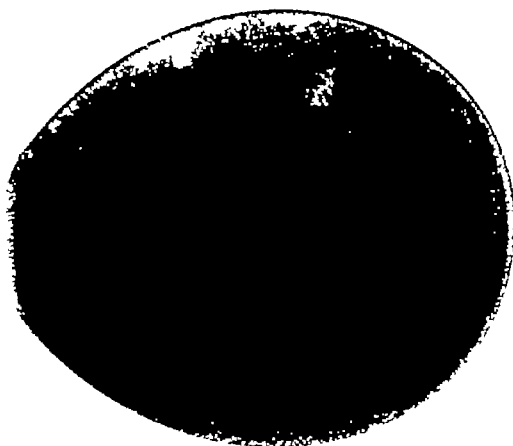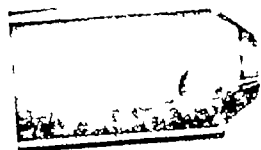
Fig 2, B
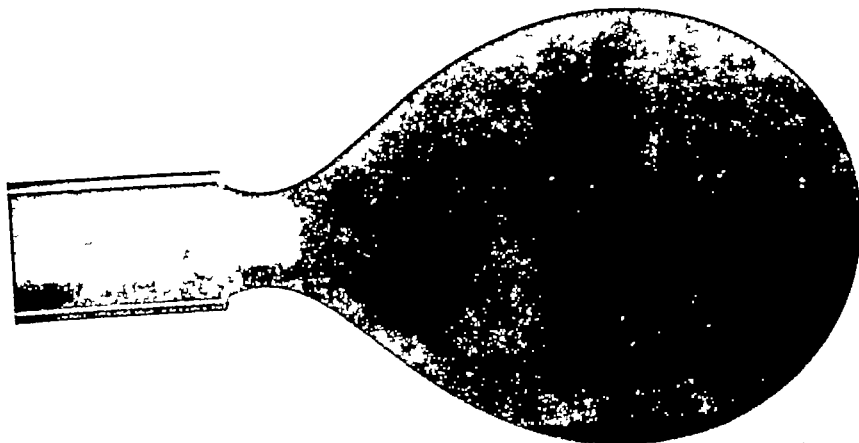
Fig 2, A

Fig 2, A, B, and C   Shows Three Steps in the Detachment of a Drop

It will be noted that a part of the drop remains hanging on the tip   The way in which the drop detaches is dependent upon the rapidity with which it forms and falls, so for an accuracy of 0 1 per cent the drop must be caused to detach itself as slowly as possible   The control rod *RCF* (fig 1) enables this to be done so accurately that it is possible with pure liquids to reduce the error to about 0 05 per cent The corrections are valid only for drops which detach slowly

minutes and those held a shorter time   Since the time used to obtain a single drop is seven minutes, it was considered advisable to use only 2 to 3 drops per determination   It may be noted that Morgan and Woodward used only a single drop

CALCULATION OF THE SURFACE TENSION

The surface tension (γ) is not, as is often assumed, proportional to the weight of the drop when a single tip is used, but it may be calculated very easily from the following equation

$$\gamma = \frac{mg}{R} \times \Gamma$$

in which m is the weight in grams of the drop which falls (as weighed

TABLE 2

Drop-weight surface tension corrections

(Factor for multiplication $= F$)   Based on the value 72 75 as the surface tension of water at 20°C R is the radius of the tip

$\frac{V}{R^3}$	F	$\frac{V}{R^3}$	F
(∞)	0 159	2 637	0 26224
5000 0	0 172	2 3414	0 26350
250 0	0 198	2 0929	0 26452
58 1	0 215	1 8839	0 26522
24 6	0 22561	1 7062	0 26562
17 1	0 23051	1 5545	0 26566
13 28	0 23522	1 4235	0 26544
10 29	0 23976	1 3096	0 2649ɔ
8 190	0 24398	1 2109	0 26407
6 662	0 24786	1 124	0 26324
5 522	0 25135	1 048	0 2617
4 653	0 25419	0 980	0 2602
3 975	0 25661	0 912	0 2585
3 433	0 25874	0 865	0 2570
2 995	0 26065		

in the gas or vapor in which it was suspended), g is the gravitational attraction, and R is the radius of the circular face of the tip

The values of F have been determined by Harkins and Brown, and are given in table 2, in which V represents the volume of the drop which falls, and $V = \frac{m}{\rho}$ in which ρ is the density of the liquid

METHOD AND RESULTS

A large part of the work consisted in evolving a method which would give constant results The data which were obtained by the procedure finally chosen will be discussed in the next section The preliminary steps in the method are listed below

TABLE 3A

The surface tension of the serum falls to a more or less constant value in the first few hours after procuring the blood

Patient		Time after procuring blood	Drop weight
		minutes	
M	{	80	0 05035
		150	0 04988
		180	0 04920
N	{	75	0 05147
		105	0 04942
		210	0 04957
O	{	240	0 05170
		270	0 05145
P	{	75	0 04865
		105	0 04808
		240	0 04832
R	{	165	0 05210
		405	0 04775
		435	0 04760
S	{	465	0 04770
		510	0 04780

Note The centrifuging was done about twenty minutes before the first determination recorded in each case, the blood being kept sealed until the centrifuging In cases N and O no centrifuging was necessary

1 Procuring the blood About 10 to 20 cc of blood is taken from the median cubital vein of either arm by means of a hypodermic syringe The syringe must be dry since water, ether, and similar substances cause hemolysis and there is a possibility that hemoglobin in solution may affect some of the measurements involved

2 Sealing the tube If the determinations cannot be made immediately, the blood should be sealed in a tube which it almost fills, so as to be in contact with as little air as possible   The tube may be stoppered with a paraffined cotton plug but is preferably sealed by the use of a minute flame   If the latter method is used, the tube should be drawn out to a fine point at one end before introducing the blood   Then only a slight heating will finish the seal   It is well not

TABLE 3B

The surface tension of samples of serum kept for varying periods of time (sealed, at 5°C) before centrifuging

Patient	First determination drop weight	Interval	Second determination drop weight	Interval	Third determination
		days		*days*	
M	0 04920	1	0 04875	2	0 04788
N	0 04942	1	0 04900	3	0 05000
O	0 05145	4	0 05235	3	0 04860
P	0 04808	1	0 04860		
Q	0 04810	1	0 04848		
S	0 04770	1	0 04932		

TABLE 3C

The lowering of the surface tension of serum on standing for several days

Patient	First determination drop weight	Interval	Second determination drop weight
		days	
D	0 06509	4	0 06260
F	0 05742	2	0 05510

to have the blood come quite to the end of the tube in this case, as it is difficult to seal glass covered with blood

3 The interval between the procuring of the blood and the observations
The interval between the procuring of the blood and the determination has been observed to be of the greatest import. In general, other things being equal, several types of variation with time occur   These will be discussed in order

a If the blood is centrifuged immediately, and the determinations

made at once, it is observed that the surface tension decreases rapidly within the first few hours to a more or less constant value   This is demonstrated by table 3A which gives all the data that concern this phenomenon

b If the blood is kept sealed, without centrifuging for several days, there is no definite change in the surface tension   This is determined by sealing several different portions of the same sample of blood in separate tubes, and opening, centrifuging, and finding the surface tension of the serum from one tube each day   Table 3B shows that the surface tension of a serum may increase or decrease or vary irregularly with the time the separate samples are kept

c After being centrifuged, the surface tension of serum decreases gradually over a period of days as is seen in table 3C

d If blood is kept for several days in a sealed tube and then centrifuged, it exhibits a rapid decrease in surface tension to a more or less constant value during the first few hours, which decrease is usually not as large as that observed in (*a*)   This is shown in table 4

4 Cleaning the apparatus   The apparatus is cleaned by sucking water through it immediately after the completion of the day's work. The serum should not be allowed to dry on the apparatus, nor should it be cleaned with alcohol, ether, or cleaning mixture as these reagents precipitate the proteins in the serum and clog the capillary

5 Determinations of density and refractive index   The density was determined by the Hammerschlag method (15), and the refractive index was determined by the use of a Bausch and Lomb Abbé Refractometer

6 The aeration of the serum   Alveolar air is blown through a long coiled tube immersed in the thermostat and into the serum through the tube mentioned above   This tube is first carefully flushed out with alveolar air from the experimenter's lungs before immersing the lower end of the short tube in the serum   When the tube is well flushed out, the distal end is dipped into the serum and alveolar air bubbled through for some time   This is done to secure equilibrium between the serum and a gas of constant composition approaching as nearly as possible that in the lungs   Saturation with alveolar air causes varying effects depending on the previous condition of the serum   In general the surface tension is lowered as is seen in table 5

TABLE 4

The surface tension of blood serum kept at 5 C for several days

Note that the tension falls to a more or less constant value in the first few hours after centrifuging

Patient	Age of blood before centrifuging	Drop weight
	days	
M	4	0 05130
	4	0 04960
	4	0 05052
	4	0 04950
C	2	0 04870
	2	0 04950
	2	0 05000
M	1	0 04830
	1	0 04925
	3	0 04787
	3	0 04816
	3	0 04763
N	1	0 05040
	1	0 04820
	1	0 04975
	1	0 04896
	4	0 04920
	4	0 05080
O	4	0 05513
	4	0 05485
	4	0 05300
	4	0 05285
	4	0 05235
	7	0 05395
	7	0 05105
	7	0 04860
P	1	0 04915
	1	0 04860
Q	1	0 04852
	1	0 04845

* For each sample the determinations are listed in order and were performed at one-half hour intervals.

THE TEMPERATURE COEFFICIENT

Due to the fact that many of the determinations were made at the Presbyterian Hospital where there is no thermostat, it was necessary to determine the temperature coefficient of the surface tension of serum   These observations were made on the blood of Patient S, an almost normal subject   The results obtained at four temperatures from 10° to 37°C are exhibited in table 6

TABLE 5

Effect of aeration with alveolar air upon the surface tension of the serum

Patient D

Drop weight before aeration	Drop weight after aeration
0 06787	0 06509
0 06743	0 06574

TABLE 6

The temperature coefficient of serum surface tension

Temperature	Drop weight	Surface tension
°C		
10	0 05340	54 26
20	0 04932	50 35
30	0 04645	47 58
37	0 04505	46 22

PATHOLOGICAL VARIATIONS

The results are not of such a nature that any definite conclusions can be drawn as to a lowering or raising of the surface tension as a result of any pathological condition   Many of the cases had several pathological factors, any one of which might have altered the surface tension   Furthermore the work of Brinkman (8) on oxalated plasma and of Zunz (9) on serum indicates that the surface tension is higher in women than in men   Until these results are confirmed or disproved, it would be best to allow a margin of error in comparing the serum surface tension of men and women

The results in relation to the pathology of the different patients are shown in table 7   In most cases the corresponding density and

refractive index are given   It is apparent that no two sets of these results run perfectly parallel   The surface tension is observed to be

TABLE 7

The surface tension of blood serum as related to the pathology of the patients from whom the blood was obtained

Temperature = 20°C

Patient	Diagnosis	Surface tension	Refractive index*	Density†
I	Normal	53 55		56
J	Normal	51 01		2
K	Normal	51 02		2
L	Diabetes	53 93		0
M	General paresis, given typhoid treatment for syphilis few days before, collapsed after spinal puncture elsewhere male, 36 years	50 41	20	42
M	A week later	49 71	38	44
C	Chronic interstitial nephritis, arterial hypertension, 7 successful pregnancies, bad vertigo albumin, epistaxis, stroke in November 1925 good renal efficiency, female, 40 years	50 43	8	20
N	Parenchymatous nephritis hemoglobin of 28 per cent, red blood cells of 2 500 000 female, 40 years	50 52	3	25
O	Sciatica chronic sacroiliac arthritis, chronic tonsillitis, female, 42 years	51 77	16	33
P	Paroxysmal tachycardia of migraine, acute cold, male 40 years	49 37	15	32
Q	Trigone cystitis, cells in kidney urines, bad cold, colored male 45 years	49 37	27	53
R	Parenchymatous nephritis, myocarditis, fat, female 39 years	48 70	16	28
S	Bad case of influenza three weeks before Wasserman+ + + + 6 years ago negative since, anemia, male, 48 years	49 57	0	8

* For simplicity the refractive index tabulated above is the number of ten thousandths the refractive index exceeds 1.390
† Similarly, the densities are expressed as ten thousandths above 1 0222 the lowest value.

low in the cases just recovering from influenza, and in neither of the two cases suffering from parenchymatous nephritis, both of whom are women, is the surface tension quite as high as the average   This

latter result would tend to agree somewhat with the results of Clausen (10)   But as Clausen gives no data in support of his conclusion, it is not known how much lowering of surface tension was obtained in parenchymatous nephritis   The high value of tension in the single diabetic case is interesting

THE RING METHOD FOR THE DETERMINATION OF THE SURFACE TENSION OF BLOOD SERUM

The ring method has been used in an enormous number of the determinations of surface tension carried out with blood serum or solutions of blood serum in water   In nearly all cases the results obtained are almost valueless, since errors of 20 per cent are not uncommon   Such results are in general incorrect from even the relative standpoint, since the errors are highly variable

1   The calculation of the surface tension   The most common error in the use of the ring method is that made in the calculation of the surface tension   The surface tension is calculated from the pull exerted by the surface of the liquid on a circular ring (of radius R) of circular wire (of radius r)   What is usually determined is the maximum pull (W) on the ring as determined by a torsion balance, or better by the proper type of analytical or assay balance   This pull is obtained by allowing the horizontal ring to come into contact with the surface and then lowering the liquid or raising the balance until the film of liquid upheld by the ring is ruptured   It is customary to consider the maximum pull (P) per centimeter of surface attached to the wire to be defined by the equation

$$P = \frac{W\,g}{4\,\pi\,R} \tag{1}$$

It is obvious that the surface tension (γ) is given by the identity

$$\gamma = P\,\frac{\gamma}{P} \quad \text{or} \quad \gamma = PF \tag{2}$$

In order that equation (2) may be used for the calculation of the surface tension of the liquid the values of the ratio $\left(\dfrac{\gamma}{P} \text{ or } F\right)$ must be known   These values might have been calculated from theory, but

this has not as yet been done   The values of this ratio were deter-
mined by Harkins, Young and Cheng (16), and more carefully later
by Harkins and Jordan (17)   The values of this fraction as now
known seem to be accurate to within 0 3 per cent, which gives consider-
able accuracy to the ring method, though it is still less accurate than
the drop weight method under the best conditions (error 0 1 per cent)

The values of the correction factor F of the equation

$$\gamma = \frac{W_g}{4 \pi R} \quad F \tag{3}$$

are given in table 8   These correction factors are valid only if the
ratio of the radius of the ring (R) to that of the wire (r) is 40, which
is the ratio most commonly used

TABLE 8

*Values of the correction factor (F) of the ring method (Harkins and Jordan) for a ring in
which R = 0 6366 cm , r = 0 01570 cm   and R/r = 40.55*

(This is the size of ring most generally used)

$\frac{R^3}{V}$	γ/P
0 43	0 988
0 5	0 973
0 6	0 954
0 7	0 938
0 8	0 925
0 9	0 914
1 0	0 905
1 1	0 898
1 2	0 892

The correction factor (F) is a function of $\frac{R^3}{V}$ in which V is the volume
of liquid lifted by the pull (W) of the ring above the level of the plane
portion of the sufficiently large surface of the liquid, so

$$V = \frac{W_g}{\rho} \tag{4}$$

in which ρ is the density of the liquid

In order to determine the surface tension the value of the total

pull (W) is first determined by the balance   From this (P) is cal-
culated by substituting the mean radius (R) of the ring in
equation (1), and the volume (V) is calculated from equation
(4)   The value of $\dfrac{R^3}{V}$ is then calculated, and from this the value of $\dfrac{F}{\gamma}$
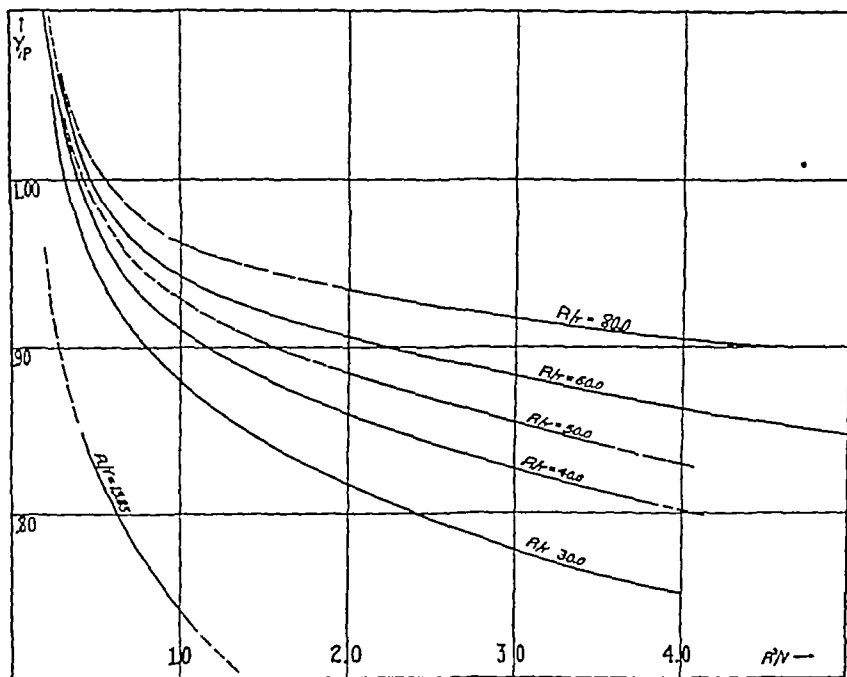
FIG 3   CORRECTION CURVE FOR THE DETERMINATION OF THE SURFACE TENSION
OF A LIQUID BY THE RING METHOD

Ordinates = values of F Abscissae = values of R^3/V The curves beginning
at the bottom represent rings for which the values of the radius (R) of the ring,
divided by the radius (r) of the wire are respectively   13 85, 30, 40, 50, 60, and 80
R is in centimeters

γ, surface tension, P, $Wg/4\pi R$, R, radius of ring, r, radius of wire, V, volume of
liquid suspended by the upward pull of the ring

is obtained by table 8   From this the surface tension γ is obtained
by equation (3)   Any simpler general method gives incorrect results

The value of the correction factor (F) varies also with the value of the

ratio $\dfrac{R}{r}$ as shown by figure 3   Table 8 gives the correction factors

which correspond with the fourth curve from the top   Any number of rings whose correction factors lie on this single curve may be used the only condition is that the radius of the ring shall be 40 times the radius of the wire, and that the ring shall be circular, plane, and, when suspended, perfectly horizontal

2 Area of the surface   The area of the surface of the liquid from which the ring is pulled should be large enough to meet the following condition   just before the detachment of the ring there must be an annular ring of surface, completely around the ring of wire, in which the liquid surface is plane   The larger the ring of wire, the larger the total surface should be   The form of surface which is most economical of liquid is that of a circle, and for *small* rings this should be about 7 cm in diameter

3 Surface tension and time   What is commonly designated as the surface tension of a liquid is the equilibrium value   It has been seen (page 267) that the time necessary to obtain equilibrium with undiluted blood serum is about seven minutes   With diluted serum this time may be more than an hour

The surface tension of blood serum cannot be obtained by allowing the serum to stand in a vessel for the time given above (seven minutes for undiluted serum) and then pulling the ring from the surface   The correct procedure is as follows   blood serum is placed in the vessel and as quickly as possible the ring is placed in contact with its surface and at once the ring is lifted to just that distance above the serum, which at the end of the correct interval to give complete adsorption (seven minutes for undiluted serum), corresponds with the beginning of instability   In other words the surface must be fully extended initially and allowed to break without further extension at the end of the proper period

In order that this may be done preliminary trials should be made to determine the proper extension, or different extensions may be applied and the pull (W) plotted against the time (t) of the rupture

4 Adjustment of the ring   It has been shown by Harkins and Jordan (17) that it is essential to adjust the ring in such a way that when it hangs from the balance its plane shall be horizontal   This has been done by obtaining the image of the ring in a plane horizontal mirror of gold or silver placed just below it

SUMMARY

1 The surface tension of normal blood serum is found to be about 52 dynes per centimeter at 20°C , and 48 dynes at 37°, and the surface tensions under certain pathological conditions have been determined On the whole the values are lower than for normal serum   The purpose of the work of the paper has been to develop proper methods for the determination of the surface tension of biological liquids, rather than to secure a large number of data

2 The worthlessness of the earlier work on the surface tension of blood serum is found to be due to (a) failure to obtain equilibrium conditions, or (b) improper methods of calculation

3 An apparatus for the determination of the surface tension of biological liquids by the drop weight method has been developed, together with a new method for the control of the drop in such a way as to give accurate results

4 The methods used in procuring the blood and in sealing up the serum are described

5 The effects upon the surface tension of the time, before and after centrifuging, have been investigated

6 Saturation of the serum with alveolar air at 20°C is found to lower the surface tension by about 2 dynes

7 Correction tables for the calculation of the surface tension by (a) the drop weight method, and (b) the ring method, are given   The almost universal failure to use these corrections has caused earlier work to be of little value

8 A method is given for the use of the ring method in the determination of the surface tension of biological liquids   The conclusion is reached that the drop weight method is somewhat superior to the ring method for such a purpose when both are properly used

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BLOOD VOLUME PRECEDING AND FOLLOWING SPLENECTOMY IN HEMOLYTIC ICTERUS AND SPLENIC ANEMIA[1]

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Very little investigative work has been published concerning the relationship of the spleen to blood volume   Barcroft, Harris, Ora-hovats, and Weiss allot to the spleen a function that correlates well with its anatomic structure   They demonstrated that the spleen acts as a reservoir in the normal resting animal, and that blood is expressed into the general circulation as a result of stimulation to a need for oxygen   The presence of a storage function is further demonstrated by the observation that carboxyhemoglobin disappears less rapidly from the spleen than from the general circulation, indicating that hemoglobin is, under normal conditions, stored in the spleen   These workers demonstrated the greater susceptibility of the splenectomized animal to carbon monoxide intoxication   Cruickshank's experiments on the cat indicate that the capacity of the spleen is from 2 to 5 per cent of the blood volume when calculated by fluctuations in hemo globin and in changes in the volume of the spleen

Observations concerning the spleen and splenectomy in relation to blood volume in man have not been found in the literature   The studies carried out previously have dealt largely with variations in the concentration of the erythrocytes and the hemoglobin, the morphology, and the chemistry of the blood   We know that splenectomy is curative in certain diseases and markedly beneficial in others, and definitely untoward effects attributable to splenectomy have not been observed in man, although there has been some question as to whether a factor of safety is removed by this operation

[1] Read by title at the meeting of the American Society for Clinical Investigation, Washington, D C , April 28, 1928

Our study was undertaken to determine (1) the blood volume and plasma volume in patients suffering from certain diseases associated with splenomegaly, (2) the effects of splenectomy on the blood and plasma volume, and (3) whether in certain forms of splenomegaly the anemia is apparent rather than actual The cases selected for this study fall into four groups

	number of cases
1 Normal subjects	74
2 Primary splenomegaly without anemia	
Preceding splenectomy	5
Following splenectomy	4
3 Hemolytic icterus	
Preceding splenectomy	11
Following splenectomy	9
4 Splenic anemia	
Preceding splenectomy	18
Following splenectomy	7

The dye method was used to determine the blood volume and plasma volume   Congo-red was employed and three to four minutes allowed for the mixing time   Determinations were made under resting conditions without breakfast (8)

It has been shown in anemia that with a decrease in the hemoglobin and cell volume there is usually an increase in plasma volume   This is doubtless a compensatory phenomenon designed to maintain an adequate volume of circulating blood   The increase of plasma varies in different forms of anemia, according to Rowntree and Brown, it is absent or slight in the anemia of glomerulonephritis with edema and in the anemia associated with myxedema and Addison's disease   In this connection we have employed the term "replacement index" to represent the ratio of the percentage increase in plasma to the percentage decrease in cell volume

For example, if the plasma volume is 61 cc for each kilogram (normal 51 2 cc) there would be an increase of 9 8 cc (19 per cent) If the cell volume is 21 cc for each kilogram (normal 36 5 cc) there would be a decrease of 15 5 cc (42 per cent)   The ratio is $\frac{19}{42} = 0 45$, the replacement index is 45   This indicates roughly that for every 100 cc loss in cell volume there is approximately an increase of 45 cc in plasma

NORMAL SUBJECTS

Dreyer and Ray have maintained that blood volume is a function of surface area rather than of body weight We have, therefore, expressed our data according to both surface area and body weight and the statistical treatment of these data has shown a slightly higher correlation of volume to surface area then to body weight in normally built subjects

The results obtained in seventy four normal adult subjects (forty-nine males and twenty five females) have been reported by Rowntree and Brown The ages varied from seventeen to sixty-three years The mean weight was 65 9 kgm The mean value for the blood volume for each kilogram of body weight was 87 7 cc (with a range of from 70 to 100 cc in 98 per cent of the cases) The mean value for the plasma volume was 51 2 cc. (with a range of from 42 to 60 cc) The mean value for the cell volume was 36 5 cc for each kilogram The mean value for the blood volume for each square meter of surface area was 3278 cc For the plasma volume it was 1920 cc for each square meter of surface area The mean value for circulating hemo globin was 13 8 grams for each kilogram of body weight, or 508 grams for each square meter of surface area The cells by hematocrit were 41 per cent[2] by the dry oxalate method, and the hemoglobin for each 100 cc was 15 7 grams These values closely approximate those obtained by Keith, Rowntree and Geraghty in their original report based on eighteen subjects Many of the normal subjects varied considerably from the accepted average weight for height and age, but could not be classified as underweight or obese When a classification of this group was made on the basis of a 10 per cent variation above or below the average standard for height and age, the mean values for the blood volume and plasma volume were only slightly different.

PRIMARY SPLENOMEGALY WITHOUT ANEMIA

Cases of primary splenomegaly without anemia, when all etiologic factors have been excluded, such as syphilis, malaria, hemolytic icterus,

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[2] In subsequent work it has been found that hematocrit values obtained by the dry oxalate method should be corrected by the addition of 3 4 per cent There fore, 41 per cent, as used here, should read 44 4 per cent

TABLE 1

Splenomegaly without definite anemia blood volume preceding and following splenectomy

Case	Sex	Age	Date	Weight (kgm)	Surface areas (sq m)	Hemoglobin (grams in each 100 cc)	Erythrocytes (millions in each cu mm)	Cells by hematocrit (per cent)	Blood Volume (cc)	Blood cc for each kilogram	Blood cc for each sq meter	Plasma Volume (cc)	Plasma cc for each kilogram	Plasma cc for each sq meter	Cells Volume (cc)	Cells cc for each kilogram	Cells cc for each sq meter	Hemoglobin Volume (grams)	Hemoglobin grams for each kilogram	Hemoglobin grams for each sq meter	Weight of spleen (grams)
1	M	49	November 23, 1922	76	1.83	13.9	4.66	42	7,480	98	4,090	4,340	57	2,370	3,140	41	1,720	1,020	13.4	557	
2*	M	25	May 25, 1923	60	1.74	15.6	5.10	41	6,330	105	3,745	3,740	62	2,210	2,590	43	1,535	987	13.1	568	
			February 22, 1925	55	1.65	16.8	3.90	46	6,870	124	4,240	3,710	67	2,290	3,160	57	1,950	1,154	20.9	700	920
3	M	36	February 2, 1926	58	1.70	15.4	5.24	45	6,290	108	3,700	3,460	60	2,040	2,830	48	1,660	969	16.6	570	
4*	M	43	June 2, 1925	62	1.68	15.0	3.82	38	5,520	89	3,260	3,430	55	2,040	2,090	34	1,220	828	13.3	493	
			June 12, 1925	62	1.68	15.0	3.93	36	5,310	85	3,150	3,395	54	2,015	1,915	31	1,135	796	12.8	475	720
5*	M	42	April 5, 1927	70	1.82	11.0	4.57	49	7,390	105	4,040	4,500	64	2,460	2,890	41	1,580	813	11.6	446	
			April 15, 1927	65	1.79	11.0	4.15	34	5,845	90	3,265	3,865	59	2,160	1,980	31	1,105	643	9.9	359	464
6*	F	26	July 22, 1927	60	1.65	15.4	3.88	43	6,390	106	3,740	3,640	60	2,170	2,750	46	1,570	984	16.4	596	800
Average values Preceding				65.2	1.75	14.2	4.68	41.0	6,602	101	3,767	3,894	59	2,224	2,708	41	1,543	923.4	13.6	526.8	
Following				60.5	1.69	14.5	3.96	39.7	6,103	101	3,598	3,652	60.0	2,158	2,451	41	1,440	894.0	15.0	532.5	

* Cases in which estimations were made following splenectomy. In case 6 the estimation was not made preceding splenectomy, and in cases 1 and 3 estimations were made preceding splenectomy only.

chronic infectious splenomegaly and various blood dyscrasias, have ordinarily been regarded as potential splenic anemia in the pre anemic stage, and the splenic enlargement has been the outstanding clinical feature

There were six cases in this group   Three were studied both before and after splenectomy   Two cases were studied preceding splenectomy and one case only after the operation   The results of these studies are shown in table 1

Comment   The blood volume preceding splenectomy was 101 cc and the plasma volume was 59 6 cc   These values are high   While the ratio of cell to plasma volume, as denoted by the hematocrit value of 41 per cent, was normal.   This state has also been disclosed in cases of chronic passive congestion and in arteriovenous fistula, conditions in which a compensatory increase in the volume of circulating blood seems to be necessary for circulatory efficiency   This leads to the possibility that in primary splenomegaly without anemia, the increased circulatory bed due to the splenomegaly and enlarged blood vessels necessitates a larger blood volume for circulatory needs

The comparison of the average values of the two groups of cases before splenectomy and after splenectomy does not show significant change in blood volume, plasma volume or in the percentage of cells by hematocrit   In case 5 with an interval of nine days, there was a decrease of 20 per cent in total blood volume and of 3 per cent in plasma volume.   Since the weight decreased 5 kgm , the changes in volume for each kilogram were not so marked   This could be ascribed to operation and loss of blood   The large blood volumes in the cases with long intervals, one year and one and a half years after splenectomy, are probably more significant, these patients have a large volume of normal blood, an unusual condition in subjects of normal build

HEMOLYTIC ICTERUS

The clinical features of hemolytic icterus are well known   The fundamental process is abnormally active hemolysis and the pathologic basis is probably chiefly concerned with increased fragility of the erythrocytes and microcytosis (Giffin)   In the diagnosis of hemolytic icterus care must be taken to exclude cirrhosis of the liver with

TABLE 2

Hemolytic icterus blood volume preceding and following splenectomy

Case	Sex	Age	Date	Weight (kgm)	Surface area (sq m)	Hemoglobin (gm in each 100 cc)	Erythrocytes (millions in each cu mm)	Cells by hematocrit (per cent)	Blood Volume (cc)	Blood cc for each kilogram	Blood cc for each square meter	Plasma Volume (cc)	Plasma cc for each kilogram	Plasma cc for each square meter	Cells Volume (cc)	Cells cc for each kilogram	Cells cc for each square meter	Hemoglobin Volume (grams)	Hemoglobin grams for each kilo-gram	Hemoglobin grams for each square meter	Weight of spleen (grams)
1	F	57	May 21, 1925	45	1 45	8 1	2 33	18	4,480	100	3,090	3,680	82	2,540	800	18	550	363	8 0	250	800
			June 2, 1925	45	1 45	8 2	2 72	24	3,510	77	2,420	2,660	59	1,840	850	19	580	288	6 4	199	
2	M	31	June 24, 1925	67	1 87	12 4	3 54	28	4,950	74	2,650	3,550	53	1,900	1,390	20	750	614	9 1	328	1,450
			July 4, 1925	62	1 80	13 6	3 84	35	6,920	109	3,840	4,500	71	2,500	2,420	40	1,340	941	15 0	522	
3	F	35	November 24, 1925	50	1 50	8 6	3 60	23	4,350	87	2,900	3,360	67	2,240	990	19	660	374	7 5	249	820
			December 8, 1925	45	1 41	12 5	4 16	33	5,160	120	3,660	3,460	80	2,450	1,700	38	1,210	644	14 3	457	
4	F	43	November 24, 1925	61	1 70	8 1	3 74	20	4,240	70	2,490	3,390	55	1,995	850	14	495	343	5 6	202	945
			December 8, 1925	54	1 61	11 7	3 74	35	5,360	99	3,330	2,950	54	1,830	2,410	44	1,500	627	11 6	389	
5*	F	22	September 15, 1925	52	1 46	6 7	3 02	20	4,320	83	2,940	3,450	66	2,350	870	17	590	289	5 6	198	1,640
6	F	6	May 24, 1926	20	0 83	8 3	2 84	19	3,060	153	3,690	2,470	123	2,975	590	30	715	254	12 0	306	500
		5	June 3, 1926	20	0 83	12 1	3 80	30	2,570	125	3,130	1,800	90	2,190	870	43	940	311	15 0	375	
7	F	4	July 31, 1925	18	0 77	8 9	3 69	24	1,560	87	2,025	1,190	66	1,545	370	20	480	139	7 7	180	203
			August 14, 1925	18	0 77	14 1	4 21	34	1,580	87	2,100	1,050	58	1,400	530	29	700	223	13 0	290	
8	M	9	July 27, 1926	23	1 00	6 8	2 57	16	2,050	89	2,050	1,720	75	1,720	330	14	330	139	6 0	139	550
			August 14, 1926	23	1 00	12 9	3 88	30	2,090	82	2,480	1,460	58	1,740	630	28	740	410	18 0	410	

9*	F	22	January 13 1927	54	1 60	7 7	2 24	24	5,210	97	3 250	3 960	73	2 475	1,250	24	775	411	7 6	257	784
10	M	14 {	February 24 1926	32	1 16	10 3	3 65	20	2 670	83	2,300	2,140	67	1 860	530	17	440	275	8 6	237	784
			March 11, 1926	31	1 16	11 2	4 24	30	3,370	109	2,900	2,360	76	2 000	1,010	32	900	377	12 0	325	
11	M	27 {	May 12 1925	54	1 60	11 4	2 93	25	4 950	92	3 090	3,710	69	2 320	1 240	23	770	564	10 5	353	1,500
			May 20 1925	50	1 52	10 2	3 93	33	6 190	123	4,020	4,140	82	2 630	2 050	40	1,390	631	12 0	415	950
Mean values†				42 7	1 38	8 86	3 10	22 3	3 795	93	2 791	2,932	73 1	2,200	836 4	21 7	597 7	359	8 1	247 7	

* In cases 5 and 9 blood volume was not estimated after splenectomy

† Mean values estimated on data preceding splenectomy only

secondary hemolytic characteristics   Splenectomy is a curative
measure   Following operation the jaundice disappears, the excessive
hemolysis ceases and, in some instances at least, the erythrocytes
become less fragile   As Moynihan stated "The spleen, if not the
exclusive cause or seat of the disease, exerts the profoundest in-
fluence on its pathogeny "

Preceding splenectomy   Eleven cases of hemolytic icterus were
studied (table 2)   The body build in this group was subject to wide
variation, none of the patients were overweight, but the younger
patients were underweight   Studies of the blood volume were made
one or two days preceding operation, and from nine to seventeen
days following operation   Anemia was present in all of the cases
The patient with a blood volume of 153 cc was the only one with a
volume above the upper range of normal, she was an emaciated girl
aged six and a half years, and according to surface area the blood
volume was less than the normal mean   According to surface area
the mean plasma volume for the entire group was 2200 cc for each
square meter, about 15 per cent higher than the normal mean

That anemia was actually present is shown by the amount of
circulating hemoglobin   The mean value for hemoglobin was 8 1
grams for each kilogram of body weight (normal 13 8 grams)   The
mean value for the cell volume was 21 7 cc for each kilogram (normal
36 5 cc), a decrease of 14 8 cc (45 per cent)   The mean value for
the plasma volume was 73 1 cc (normal 51 2 cc), an increase of 21 9
cc (43 per cent)   The ratio was, therefore, $\frac{43}{45} = 0$ 95   This replace-
ment index of 95 is high, almost twice that seen in primary and simple
secondary anemia

The hemoglobin in grams and the blood volume when separately
plotted against the weight of the spleen did not correlate   The cell
volume calculated for each kilogram of body weight plotted against the
weight of the spleen did not show definite correlation   There was a
fairly high correlation of 0 87 between plasma volume and weight of
the spleen

Following splenectomy   Estimations were made from nine to seven-
teen days following splenectomy in nine of the eleven cases (table 2)
The plasma volume according to body weight and surface area
showed a smaller percentage variation since the body weight decreased

after operation   The total hemoglobin volume increased in all but
one case, the range of increase being from 57 to 327 grams, averaging
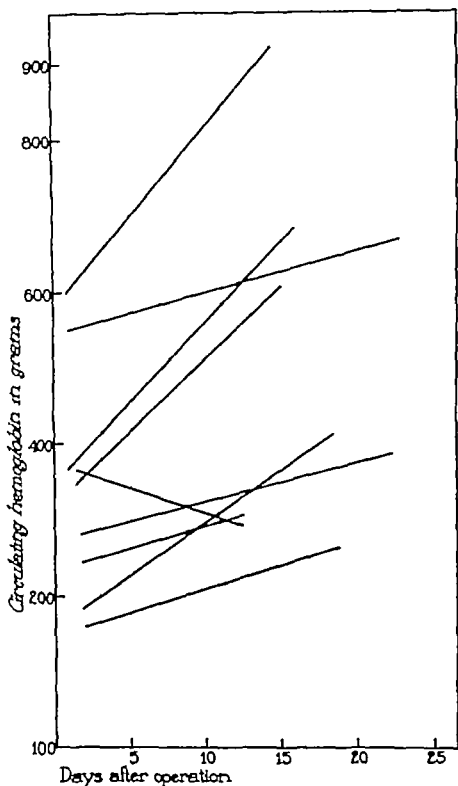
FIG 1 CHANGES IN THE CIRCULATING HEMOGLOBIN IN CASES FOLLOWING
SPLENECTOMY FOR HEMOLYTIC JAUNDICE

183 grams (fig 1)   The percentage of cells by hematocrit and the
number of erythrocytes increased in every case, and the hemoglobin in
grams for each 100 cc showed a significant increase in all but two cases

Comment The status of the blood volume in hemolytic icterus, according to mean values, is that of decreased cell volume with normal blood volume   The cell volumes and the hemoglobin volumes according to body weight and surface area were decreased, which is indicative of the existence of an actual rather than an apparent anemia With the exception of one case, that of a child, the blood volume conformed closely to the normal range, which is 70 to 100 cc for each kilogram   The plasma volume, however, was greatly increased, the increase amounting to a mean of 21 9 cc , 43 per cent for each kilogram above the normal mean   The replacement index was high, 95, in other words, when compared to normal values the increase of plasma to replace the decrease in cells was almost complete   This indicates that in hemolytic icterus the replacement of the diminished cell volume by plasma is effective and adequate, much more so, in fact, than in primary anemia and simple secondary anemia without splenomegaly

The increase in the blood volume and cell volume following splenectomy in cases of hemolytic icterus is most striking and correlates well with the clinical improvement following operation   In every case but one the cell volume increased significantly within three weeks and the actual volume of hemoglobin showed comparable changes, the increase averaging 183 grams   In case 2 (table 2) the increase in the blood volume was more than a third of the preoperative value, and the actual increase of hemoglobin was 327 grams in ten days, or over 30 grams a day   This increase of hemoglobin represents only the demonstrable increase of pigment and does not include the amount necessary to compensate for that which is normally destroyed   Figures for the normal amount of hemoglobin formed daily in man are not available Whipple, Robscheit and Hooper have shown that in the dog after unit hemorrhages, the amount of hemoglobin regenerated depends largely on the type of diet   In one experiment with beef heart diet, 43 grams of hemoglobin were formed daily over a seven-day period

The rate of recovery from anemia after splenectomy is not correctly shown in the concentration values for hemoglobin and erythrocytes For example, in case 2 (table 2) following operation there was an increase of about 9 per cent in the grams of hemoglobin in each 100 cc and a questionable increase in the number of erythrocytes   The

increase in the blood volume, however, was 1970 cc (40 per cent) half of which was cells   The increase in the actual volume of hemoglobin was 327 grams, or about 50 per cent   The plasma volume increased 940 cc while the cell volume increased 1030 cc , that is, cells increased in greater proportion than plasma, thus affecting the ratio of cells to plasma

Hooper, Robscheit and Whipple have shown that in the recovery from experimental anemia, the increased plasma volume caused by the anemia gradually disappears with the regeneration of cells   In the recovery from anemia in hemolytic icterus, the plasma volume decreased in five cases with the increase of cells, and increased in four cases

The status of the blood volume as indicated by the mean value in a series of eleven cases of hemolytic icterus preceding splenectomy is that of decreased cells with normal volume   The anemia of hemolytic icterus is an actual rather than an apparent anemia and as the plasma volume increases to cause a slightly high blood volume a small amount of dilution occurs   The blood volume is maintained within a normal range since the replacement of cell volume by plasma is almost complete, as shown by a replacement index of 95   Following splenectomy there is an immediate rapid increase in the cell volume and hemoglobin volume which is not adequately demonstrated by the ordinary clinical estimations for determining the concentration of hemoglobin and erythrocytes

SPLENIC ANEMIA

Splenic anemia is a useful term to indicate a poorly defined clinical syndrome rather than a disease entity, and the diagnosis is chiefly made by exclusion   Cases included in this group have two features in common   primary splenomegaly, and a secondary type of anemia   Cases of questionable diagnosis are always present in a group of cases classified as splenic anemia, and the difficulties of arriving at conclusions concerning observations on the blood volume are correspondingly increased, especially in those cases in which secondary hepatic cirrhosis is present

Preceding splenectomy   Estimations of blood volume were made preceding splenectomy in eighteen cases classified as splenic anemia,

TABLE 3

Splenic anemia: blood volume preceding* and following splenectomy

Case	Sex	Age	Date	Weight (kgm)	Surface area (sq m)	Hemoglobin (grams in each 100 cc)	Erythrocytes (millions in each cu mm)	Cells by hematocrit (per cent)	Blood Volume (cc)	Blood cc for each kilogram	Blood cc for each square meter	Plasma Volume (cc)	Plasma cc for each kilogram	Plasma cc for each square meter	Cells Volume (cc)	Cells cc for each kilogram	Cells cc for each square meter	Hemoglobin Volume (grams)	Hemoglobin grams for each kilogram	Hemoglobin grams for each square meter	Weight of spleen (grams)
1	F	13	November 2, 1923	53	1.70	11.8	3.50	29	5,780	109	3,610	4,100	77	2,560	1,680	31	982	681	13	400	
2†	M	41	December 11, 1926	74	1.96	8.3	4.34	30	8,300	112	4,230	5,800	78	2,960	2,500	34	1,288	688	9	352	300
3	F	31	July 17, 1925	69	1.77	7.6	3.50	26	4,630	67	2,620	3,430	50	1,940	1,200	17	677	338	5	190	400
4†	M	47	January 27, 1926	80	1.93	9.2	3.33	26	8,510	107	4,410	6,320	79	3,270	2,190	27	1,135	783	9	405	
			February 20, 1926	70	1.82	11.2	3.00	25	7,280	104	4,000	5,450	77	2,950	1,830	26	1,010	815	11	448	1,460
5	F	23	June 23, 1926	44	1.30	8.5	3.43	32	4,720	107	3,630	3,210	73	2,470	1,510	34	1,160	401	9	308	
			January 21, 1927	40	1.31	9.5	3.70	26	3,955	98	3,020	2,930	73	2,235	1,025	25	788	375	9	286	700
6†	M	30	October 14, 1926	87	2.02	10.9	3.96	29	7,460	86	3,690	5,300	61	2,630	2,160	24	1,040	804	9	398	1,440
7†	M	35	November 4, 1926	72	1.92	10.7	3.70	30	7,345	102	3,845	5,140	71	2,680	2,205	30	1,160	786	10	410	
			May 31, 1927	72	1.87	7.9	4.32	30	7,350	102	3,910	5,140	71	2,750	2,210	31	1,181	580	8	310	1,160
8†	F	28	July 21, 1927	62	1.65	11.6	4.29	34	6,380	103	3,870	4,210	68	2,550	2,170	35	1,321	741	12	450	
			August 12, 1927	57	1.59	12.1	4.32	35	5,780	101	3,635	3,710	65	2,330	2,070	36	1,380	699	12	439	1,140
9	F	29	September 4, 1927	49	1.46	7.6	3.18	25	4,750	97	3,250	3,560	73	2,440	1,190	24	815	362	7	247	
			September 19, 1927	45	1.42	6.6		27	4,570	101	3,220	3,330	74	2,345	1,240	27	885	302	6	213	1,000
10	F	34	June 3, 1927	59	1.65	12.8	4.04	42	6,400	108	3,850	3,710	63	2,240	2,690	45	1,670	819	14	511	
			June 20, 1927	53	1.60	12.6	3.42	37	5,665	106	3,545	3,565	67	2,225	2,100	40	1,400	714	13	446	986

11	F	40	May 10, 1927	56	1 62	11 1	3 69	37	5,860	104	3,620 3 690 66	2,270 2,170	38 1 340 650	12 0 401	1,000
12	F	42	September 14 1927	53	1 59	12 0	3 96	32	6,010	113	3,784 4,090 77	2,570 1,920	36 1,207 721	13 0 453	
13†	M	49	September 22 1927	85	2 00	5 6	2 71	21	7,120	84	3,560 5,620 66	2,800 1 500	18 750 399	5 0 197	800
14	M	55	February 28 1928	70	1 76	7 5	2 79	23	6 500	93	3 700 5,000 71	2,840 1 500	22 860 486	7 0 276	
14	M	55	March 15 1928	63	1 69	9 3	3 42	26	5 539	88	3,270 4,090 65	2,420 1,440	23 850 514	8 2 304	1,480
15†	F	18	December 8, 1926	60	1 65	10 0	3 86	31	5 930	99	3 590 4 090 68	2,480 1,840	30 1 115 593	9 9 359	1,800
16	F	55	March 26, 1925	54	1 49	14 1	3 62	33	4,030	75	2,710 2,700 50	1 810 1,330	25 900 568	10 5 381	
17	F	59	June 21 1927	51	1 49	8 1	3 41	26	5 750	112	3 860 4,250 83	2,840 1,500	30 1,006 466	9 0 312	
18†	M	26	October 22, 1925	73	1 92	11 2	3 68	32	5 760	79	3,000 3 920 53	2 040 1,840	25 958 645	8 8 335	1,170
Mean values‡				64 2	1 72	9 8	3 70	30	6,194 5	97	3,600 4 888 68	62 512 1,855	30 1 078 594 4	9 7 355 6	
Average values§				57 1	1 62	10 3	3 59	29	4 5,732	0 100	0 3,505 4,031 70	3 2,455 701	30 1,068 600 7	10 3 363 7	

* In cases for which only one row of values is given, estimations were made preceding splenectomy only
† Banti's disease.
‡ Mean values estimated on data preceding splenectomy only
§ Average values estimated on data following splenectomy only (cases 4, 5, 6, 8 9, 10 and 14)

TABLE 3

Splenic anemia. Blood volume preceding* and following splenectomy

Case	Sex	Age	Date	Weight (kgm)	Surface area (sq m)	Hemoglobin (grams in each 100 cc)	Erythrocytes (millions in each cu mm)	Cells by hematocrit (per cent)	Blood Volume (cc)	Blood cc for each Kilogram	Blood cc for each square meter	Plasma Volume (cc)	Plasma cc for each Kilogram	Plasma cc for each square meter	Cells Volume (cc)	Cells cc for each Kilogram	Cells cc for each square meter	Hemoglobin Volume (grams)	Hemoglobin grams for each Kilogram	Hemoglobin grams for each square meter	Weight of spleen (grams)
1	F	43	November 2, 1923	53	1 70	11 8	3 50	29	5,780	109	3,610	4,100	77	2,560	1,680	31	982	681	13	0.400	
2†	M	41	December 11, 1926	74	1 96	8 3	4 34	30	8,300	112	4,230	3,800	78	2,960	2,500	34	1,288	688	9	0.352	300
3	Γ	31	July 17, 1925	69	1 77	7 6	3 50	26	4,630	67	2,620	3,430	50	1,940	1,200	17	677	338	5	0.190	400
4†	M	47	January 27, 1926	80	1 93	9 2	3 33	26	8,510	107	4,410	6,320	79	3,270	2,190	27	1,135	783	9	0.405	
			February 20, 1926	70	1 82	11 2	3 00	25	7,280	104	4,000	5,450	77	2,950	1,830	26	1,010	815	11	6 448	1,460
5	Γ	23	June 23, 1926	44	1 30	8 5	3 43	32	4,720	107	3,630	3,210	73	2,470	1,510	34	1,160	401	9	0.308	
			January 21, 1927	40	1 31	9 5	3 70	26	3,955	98	3,020	2,930	73	2,235	1,025	25	788	375	9	3 286	700
6†	M	30	October 14, 1926	87	2 02	10 9	3 96	29	7,460	86	3,690	5,300	61	2,630	2,160	24	1,040	804	9	2 398	1,440
7†	M	35	November 4, 1926	72	1 92	10 7	3 70	30	7,345	102	3,845	5,140	71	2,680	2,205	30	1,160	786	10	9 410	1,160
			May 31, 1927	72	1 87	7 9	4 32	30	7,350	102	3,910	5,140	71	2,750	2,210	31	1,181	580	8	0.310	
8†	Γ	28	July 21, 1927	62	1 65	11 6	4 29	34	6,380	103	3,870	4,210	68	2,550	2,170	35	1,321	741	12	0.450	
			August 12, 1927	57	1 59	12 1	4 32	35	5,780	101	3,635	3,710	65	2,330	2,070	36	1,380	699	12	0.439	1,140
9	Γ	29	September 4, 1927	49	1 46	7 6	3 18	25	4,750	97	3,250	3,560	73	2,440	1,190	24	815	362	7	3 247	
			September 19, 1927	45	1 42	6 6		27	4,570	101	3,220	3,330	74	2,345	1,240	27	885	302	6	7 213	1,000
10	I	34	June 3, 1927	59	1 65	12 8	4 04	42	6,400	108	3,850	3,710	63	2,240	2,690	45	1,670	819	14	0.511	
			June 20, 1927	53	1 60	12 6	3 42	37	5,665	106	3,545	3,565	67	2,225	2,100	40	1,100	714	13	4 446	986

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plasma volume and the weight of the spleen   It has been suggested
that in the cases of enlarged spleen and a presumably active reticulo-
endothelial system the dye is removed from the blood with abnormal
rapidity   Information on this point has been obtained by the following
experiments

1 In two cases of hemolytic icterus volume determinations were
made on the operating table just prior to removal of the spleen
Twenty to thirty minutes after the injection of the dye, samples of
blood were taken from the splenic artery, splenic vein and spleen
pulp   It was found that the concentration of the dye in the three
samples was exactly the same   These experiments seem to demon-

TABLE 4

Percentage of variation in the plasma volume within six minutes after injection of the dye

Case	Time after injection			
	2 minutes	3 minutes	4 minutes	6 minutes
1	100	100	100	100
2		100	101	107
3	100	100	101	102
4	100	100	100	104
5	100	99	100	96
6	100	97	100	100

* The two-minute determination is taken as 100 per cent.

strate that the dye is not abnormally removed by the cells of the
spleen in hemolytic icterus

2 The rate of disappearance of the dye from the blood was ascer-
tained by determining the plasma volume at periods of two, three,
four and six minutes in a large series of normal subjects and in six
cases of splenic anemia (table 4), demonstrates that an increased or
abnormal rate of disappearance of the dye was not present in the cases
of splenic anemia   Moreover, dye was not excreted in the urine
within one hour after injection, as has been demonstrated in certain
cases of nephrosis

The effect of anemia on the increase in plasma volume was con-
sidered   Only rarely in cases of secondary anemia were high plasma
volumes found   In thirty cases of primary and secondary anemia there

seven of the Bantı type (table 3), It will be noted in table 3 that in the seven cases of the Bantı type there was approximately the same high blood volume as that in the cases without advanced hepatic cirrhosis

The body build of this group of patients was fairly uniform None was obese or emaciated The mean figure for body weight was 64 2 kgm

Following splenectomy In seven of the cases observations were made both before and after splenectomy Estimations of blood volume were made from two to three weeks after operation (table 3)

Comment The mean value for the blood volume according to the body weight and surface area is increased in splenic anemia approximately 10 per cent above the normal This increase is largely due to plasma volume since the cell volume is reduced 18 per cent The mean value for the plasma volume according to weight and area is approximately 30 per cent above normal The plasma volume is much higher in splenic anemia than in the chronic secondary types of anemia and in pernicious anemia with comparable hemoglobin and cell values This fact suggests the question whether the anemia is actual or whether dilution factors play a rôle causing apparent anemia The circulating hemoglobin in grams for each kilogram preceding splenectomy was 9 7 grams, or for each square meter of surface area 355 6 grams compared with a normal of 13 8 grams and 508 grams, respectively This would indicate that actual anemia exists which is diluted by the abnormally high plasma volume The anemia is due both to dilution and to actual loss of hemoglobin The plasma not only replaces the lost cell volume but increases to a point beyond the original blood volume The replacement index is very high, as is demonstrated by the following calculation A mean cell volume of 30 cc for each kilogram, compared with a normal of 36 5 cc , is 6 5 cc , 17 8 per cent, less than normal, the mean value for the plasma volume of 68 6 cc (normal 51 2 cc) is 17 4 cc , 34 per cent, above normal The replacement index would then be 191 ($\frac{34}{178}$ = 1 91) The replacement index as demonstrated in sixteen cases of secondary anemia was 42 and in ten cases of pernicious anemia was 30

The reason for the increase in plasma volume in cases of splenic anemia is not clear although there is a close correlation between the

plasma volume and the weight of the spleen   It has been suggested that in the cases of enlarged spleen and a presumably active reticulo-endothelial system the dye is removed from the blood with abnormal rapidity   Information on this point has been obtained by the following experiments

1 In two cases of hemolytic icterus volume determinations were made on the operating table just prior to removal of the spleen Twenty to thirty minutes after the injection of the dye, samples of blood were taken from the splenic artery, splenic vein and spleen pulp   It was found that the concentration of the dye in the three samples was exactly the same   These experiments seem to demon-

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* The two-minute determination is taken as 100 per cent

strate that the dye is not abnormally removed by the cells of the spleen in hemolytic icterus

2 The rate of disappearance of the dye from the blood was ascertained by determining the plasma volume at periods of two, three, four and six minutes in a large series of normal subjects and in six cases of splenic anemia (table 4), demonstrates that an increased or abnormal rate of disappearance of the dye was not present in the cases of splenic anemia   Moreover, dye was not excreted in the urine within one hour after injection, as has been demonstrated in certain cases of nephrosis

The effect of anemia on the increase in plasma volume was considered   Only rarely in cases of secondary anemia were high plasma volumes found   In thirty cases of primary and secondary anemia there

was a moderate increase in the relative volume of plasma with mean values of 60 and 58 cc , respectively   This would indicate that the anemia is probably not the sole factor in the production of the high plasma volume in splenic anemia   It was demonstrated in the series of cases reported in the first portion of this paper that cases of spleno-megaly without anemia also show a high plasma volume   It is possible, then, that a high plasma volume may be one of the funda-mental changes in cases of splenic anemia, and that it may precede the development of anemia
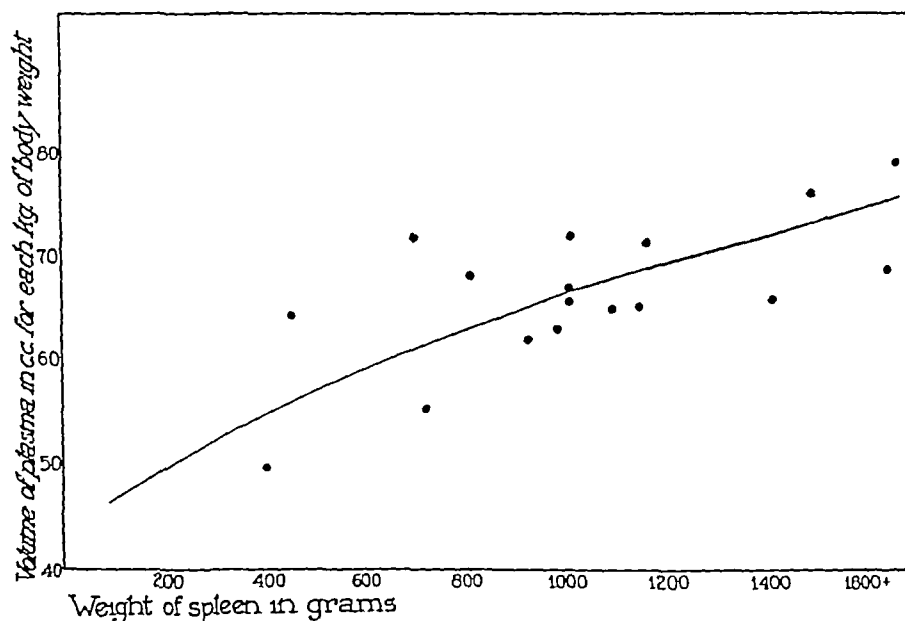
FIG 2   PLASMA VOLUME AND WEIGHT OF SPLEEN IN SPLENIC ANEMIA

Following splenectomy a decrease in the total volume of the circu-lating blood occurred in every case   This decrease was slight in one instance but definite in all the others   The maximal decrease was 1230 cc and the average was 656 cc   The decrease in the total blood volume was more constant and marked than that occurring in the relative blood volume for each kilogram because of variations in body weight   On the basis of surface area a decrease occurred in all but one case   The plasma volume likewise decreased in every instance, with a range of decrease from 145 to 910 cc , the average decrease being

442 cc   Slight increases and decreases occurred according to body weight   The cell volume decreased in five of the seven cases, but was significant in only three cases   In only two cases did the cell volume show an increase   The total amount of circulating hemoglobin in grams showed a decrease in five of the seven cases, averaging 50 grams

The total blood volume for each kilogram of body weight was charted against the weight of the removed spleen   The curve shows a rough correlation between these factors   It is apparent that larger volumes of blood occurred in the cases in which the spleen was largest. A closer correlation is brought out when the plasma volume for each kilogram is plotted against the weight of the spleen   The curve approximates a straight line (fig 2)   Between the cell volume and the weight of the spleen definite correlation could not be demonstrated

The moderate reduction in the blood volume after splenectomy in cases of splenic anemia would, at first thought, be attributed to loss of blood   The studies in these cases were made two and three weeks following operation, this interval would ordinarily be regarded as sufficient for adequate blood regeneration   There is great variation in the amount of blood lost at operation, in some cases it is negligible and in others large   There does not seem to be any correlation between the amount lost at operation and the decrease in blood volume. Likewise, the drop in the volume of blood after operation consists largely of plasma, indicating a readjustment following splenectomy A relationship could not be established between the reduction in blood volume and plasma volume and the weight of the spleen, in fact, the patient (case 5, table 3) with the smallest spleen, showed the greatest decrease in blood volume   Surprisingly small amounts of blood were recovered from the enlarged spleens, the amount ranged from 100 to 500 cc   Either the contraction of the spleen eliminates much of the blood before it is removed, or its actual capacity is reduced, possibly because of fibrosis   One would infer from these observations that loss of blood due to removal of the spleen, although it may affect the results to a certain degree, does not account for all of the reduction in volume of blood, nor could it account for the disproportionate loss in the plasma volume and the relatively high plasma volumes remaining after splenectomy

Summarizing these results in connection with splenic anemia, it has

apparently been shown (1) the plasma volume, and therefore the total blood volume, is high according to body weight in spite of a decreased volume of cells, (2) the increased plasma volume estimation is not due to an abnormal affinity of the cells of the spleen for the dye, to loss of the dye in the urine, or to an abnormally rapid rate of disappearance from the blood, (3) while the existence of anemia is usually a significant factor in the production of the high plasma volume, it is not the important or sole factor as it seems to be in cases of secondary anemia and pernicious anemia, (4) the enlarged spleen is probably an additional factor in the increase of plasma volume and total blood volume, even in splenomegaly without anemia the plasma volume is slightly increased, and (5) a fairly high correlation exists between the weight of the spleen and the plasma volume for each kilogram

SUMMARY

The figures obtained in blood volume studies in seventy-four normal adult subjects reported elsewhere by Rowntree and Brown are approximately as follows for both sexes, mean value for the blood volume for each kilogram of body weight, 87 7 cc , plasma volume for each kilogram, 51 2 cc , cell volume, 36 5 cc , circulating hemoglobin for each kilogram of body weight, 13 8 grams, and for each square meter of surface area, 508 grams, hemoglobin for each 100 cc , 15 7 grams, and cells by hematocrit, 41 per cent   On the basis of each square meter of surface area the values are approximately as follows volume of blood, 3278 cc , volume of plasma, 1920 cc , and volume of cells, 1358 cc

In cases of primary splenomegaly without anemia the mean value for the blood volume was increased to 101 cc for each kilogram, the plasma volume was increased to 59 6 cc , and the ratio of the cells to plasma was normal   After splenectomy there was no significant change in the blood volume or in plasma volume, the values still remaining above normal

In hemolytic icterus the blood volume was normal and the cell volume decreased, the replacement of the diminished cell volume by plasma was complete   After splenectomy there was an increase in blood volume and a very striking increase in the volume of cells and hemoglobin   These increases were much greater than were indicated

In splenic anemia blood volume was increased and cell volume decreased   An increase in the plasma volume was found which could not be accounted for solely on the basis of anemia   The anemia was due both to dilution and actual loss of hemoglobin   Following splenectomy a moderate decrease, which was largely in the plasma volume, occurred in the total blood volume, yet the increased plasma volume persisted   A high correlation has been demonstrated for the relative plasma volume and the weight of the removed spleen

Most of these observations were made within three weeks of the time of operation   The number of observations made at longer intervals subsequent to splenectomy have been too few to warrant discussion

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PROCEEDINGS OF THE FIRST MEETING OF THE CEN'
SOCIETY FOR CLINICAL RESEARCH HELD IN
CHICAGO, ILL , NOVEMBER 23, 1928

The Influence of Respiration on Venous Pressure By O O MEYER, M
invitation), and WM. S MIDDLETON, M.D , Madison, Wis

Movement of blood into the thorax is favored by the increased negat:
intrapleural pressure on inspiration. Accordingly venous pressure in the
sible peripheral veins of the forearm falls on inspiration With the initi
expiration and the coincident fall of intrapleural negative pressure, pe
venous pressure increases Under ordinary circumstances in the normal ind
these changes are too insignificant to warrant clinical attention Howe'
theoretical considerations stated develop a practical importance when
ances of respiratory rhythm occur, as in the induction of anesthesia and (
Stokes respiration The physical exertion, respiratory arrest and probable
dioxide increase of the induction stage of general anesthesia lead to sha
of the venous pressure The period of hyperpnoea of Cheyne-Stokes res]
invariably induces a fall of venous pressure whereas an increase atter
apneic phase

Led by these observations to seek a practical application as a dynamic
right ventricular integrity, a series of studies was pursued relative to the
pressure response to the Valsalva and to the Mueller tests Briefly sta
results confirm the concensus of opinion as to the rise of venous pressure w
Valsalva experiment, but refute the belief that the Mueller test leads to a
tion of venous pressure In both instances in normal subjects a rise is n
whose level apparently bears a direct relation to the duration of the exp
and to the energy of the resisted respiratory effort Obviously the resul'
the Valsalva experiment have a definite bearing on the circulatory fata!
child birth and on the so-called "bed pan" deaths in cardiac cases

Further developments of this attack led to the application of the V
experiment to the various stages of cardiac incompetency It is believ
more prompt and higher early elevations of the venous pressure may be anti
in event of right ventricular weakness, but the resisted expiratory effort
be maintained under this circumstance The ultimate level of venous |
in decompensated individuals under the conditions of the Valsalva exp
therefore does not approach that noted in normal controls Hence a pr
value attaches to the study of the venous pressure reaction of such case:
Valsalva experiment.

The Cardiodynamic Effects of Stenosis of the Aorta on the Pulmonary Circuit and Right Heart By LOUIS N KATZ, M D , and MORTIMER L SIEGEL, M D (by invitation), Cleveland, Ohio

An attempt was made in the present investigation to determine whether or not the left ventricle can counteract the effects of an impediment to the onward flow of blood and prevent back pressure effects in the pulmonary artery and right heart A marked constriction was applied at the root of the aorta and its effect on the pressure pulses in the left auricle, pulmonary artery, and right ventricle was followed in optically recorded curves

Obstruction of the coronary return flow to the right heart had little or no effect on the curves showing that changes produced were due to an increased coronary flow

Our results indicate that stenosis of the root of the aorta leads to a damming back of blood in the pulmonary artery and right ventricle sufficient to raise the pressure within them The left ventricle, left auricle, and pulmonary veins are apparently inadequate to accommodate the excess

Observations on the Rumpel-Leede Phenomenon By JOHNSON McGUIRE, M D (by invitation), Cincinnati, Ohio

The occurrence of petechial cutaneous hemorrhages following artificially induced venous stasis has been described in various diseases

Hecht, in 1907, published an account of petechiae observed in children ill with scarlet fever These hemorrhages developed when loose folds of skin were picked up and held lightly between the fingers

During an epidemic of scarlet fever in 1911, Rumpel and Leede noted the appearance of similar hemorrhages following the application of a tourniquet They came to the conclusion that the test was specific for scarlet fever

Stephen and later Weissman studied skin hemorrhages in a fairly large series of miscellaneous cases Using the cuff of an ordinary sphygmomanometer stasis was induced by raising the pressure to 70 mms of Hg and maintaining this level for five minutes

Petechial hemorrhages occurred regularly in subacute bacterial endocarditis, frequently in cases of hypertension, occasionally in rheumatic fever, scurvy and acute nephritis, never in tuberculosis, staphylococcus infections, pernicious anemia, or diabetes

Further studies have been made on patients with a variety of diseases and on a number of normal controls

Description of method The result in groups of individuals suffering from 25 diseases are recorded in table 1

The results in 50 normals in table 2

An examination of the results in table 1, shows a markedly positive result with the tourniquet test in all cases of subacute bacterial endocarditis Inconstant findings occurred in the other diseases

In the hypertensive group Weissmann obtained an extremely high percentage of positive reactions, while this series shows a number of negatives with equally high blood pressures   The probable explanation of this discrepancy is the fact that practically every one will give a positive result when the pressure is maintained at 100 mm for five minutes   To produce what he considered appropriate stasis Weissmann used pressures just below the diastolic levels in the cases of hypertension

Table 2 shows that approximately one third of normals gave slightly positive results

Summary and conclusions   The occurrence of petechiae following venous stasis has been studied in 25 diseases and in a group of normal controls   The results are given in two tables

In the few cases of subacute bacterial endocarditis examined, the result was uniformly strongly positive and suggests the sign may prove of differential diagnostic value

The frequency of a slightly positive reaction in normals suggests that only markedly positive results be considered significant

The Effects of Temporary Occlusion of the Coronary Arteries   By DON C SUTTON, M.D (by invitation), Chicago, Ill

1 A method for the study of the effect of temporary occlusion of the coronary arteries in unanesthetized dogs has been developed

2 Temporary occlusion of the coronary arteries in the dog produces pain which is abolished by removal of the left stellate ganglion, by destruction of the perivascular nerves, but not by severance of the vagi

3 The visceral pericardium is not sensitive to pain

The Significance of Electrocardiograms Showing Left Axis Deviation with Inverted T I and Upright T III and Q R S of Normal Duration   By DREW LUTEN, M D , and EDWARD GROVE, M D (by invitation), St. Louis, Mo

Experimental evidence (Wilson and Herrmann) indicates that electrocardiograms of this type result from impairment of conductivity in the right limb of the A V bundle   Two examples of supporting clinical evidence are presented   Our conception of the cause of this defect refers it to coronary disease

Such records in our files (237 in number) were found to be confined almost exclusively to patients exhibiting either definite or presumptive arterial disease. Heart disease, when it occurred (56 per cent of cases), was almost invariably of types referable, wholly or in part, to coronary involvement.

The higher incidence of such defect in the right limb is readily explained by the coronary distribution to the two divisions of the A V bundle (Gross)

Emaciation, Anemia, Betany, Chronic Diarrhea and Malabsorption of Fat   A Nutritional Disturbance in Adults which Resembles Celiac Disease and Sprue   By WILLIAM H HOLMES, M.D (by invitation), Chicago, Ill.

The article deals with a group of six cases in which emaciation, anemia, tetany

and chronic diarrhea were the dominant symptoms In certain instances the emaciation amounted to a loss of 50 per cent of the usual body weight The anemia resembled Addisonian anemia but differed in that there were no signs of postero lateral sclerosis or of an acidity Subjective symptoms consisting of tingling and numbness of the extremities were due to tetany and disappeared immediately with restoration of a normal blood calcium The emaciation and anemia appear to be due to malabsorption of food Starches and fats are poorly utilized A high protein diet was well tolerated and did not aggravate the tetany A markedly dilated and redundant colon was present in all cases Dietetic control and restoration of normal mineral balance resulted in a cure in two instances and improvement in the remainder

Chemical Studies of Patients with Non-Tropical Sprue By PAUL STARR, M D , Chicago, Ill

This report consists of (1) a detailed account of the results of diet, thyroid, parathyroid and calcium medication on the metabolic rate, weight, blood calcium and stools of a patient with non-tropical sprue observed from May 1926 to November 1928 (2) the chemical data of calcium balance metabolism experiments on a second patient on low fat, high protein diet, normal high fat diet, and normal low fat diet, covering twenty-four three day periods during five months observations, (3) the effect of gastric digestion on the blood calcium in normal patients with a few observations on patients with sprue, consisting of a tabulation of the change in the blood calcium during a fractional meal in twenty instances The suggestions in regard to the calcium metabolism of sprue to be drawn from these studies are as follows (1) Chronic latent tetany is present in non-tropical sprue (2) it may become acute with extreme depression of the blood calcium, (3) negative calcium balance could not be detected on low fat diets, (4) negative calcium balance was found on a high fat diet during diarrhea, (5) treatment calculated to correct this is beneficial, (6) there is an associated depressed basal metabolic rate, and (7) benefit from raising it by thyroid administration, (8) there is a slight but definite depression of the blood calcium during the gastric digestion of a fractional test-meal, (9) this is not dependent upon acid secretion

Hyperparathyroidism By HAROLD BULGER, M D , HENRY H DIXON, M D (by invitation) and DAVID P BARR, M D , St Louis, Mo

We have studied a patient presenting multiple giant-cell bone tumors, hypotonicity of muscles, bilateral nephrolithiasis, hypercalcemia and abnormal tendency to excrete calcium in the urine and a parathyroid tumor

The removal of the parathyroid tumor caused severe and almost fatal tetany, hypocalcemia, change in the size of the bone tumors and abnormal tendency to the retention of calcium

The symptoms of this patient closely resemble many cases which are scattered

through the literature but which have never been sufficiently defined It is suggested that these are cases of true hyperparathyroidism

The Instance of Peptic Ulcer as Determined Post Mortem By A J MILLER, M D , Louisville, Ky

Careful examination of the stomach and duodenum in patients over fifteen years of age coming to autopsy at the University Hospital in Omaha, Nebraska, over a period of two years revealed an instance of peptic ulcer of 18 8 per cent There are 202 cases in the series Thirty-eight of them had definite evidence of ulceration in the stomach or duodenum, five of these, or 13 1 per cent were diagnosed clinically, thirty three, or 86 9 per cent, were not recognized Twenty-one, or 55.2 per cent, were entirely healed lesions, and of these, fourteen, or two-thirds of the healed lesions, were in the duodenum Thirteen, or 34.3 per cent, were active lesions, and of these, three, or approximately one-third, were in the duodenum In four, or 10 5 per cent there were active and healed lesions all of which were in the stomach

The instance of peptic ulcer is much greater than commonly believed Those in the duodenum heal much more frequently than those in the stomach

A Study of Pulmonary Lesions Among Children and Young Adults By J A MYERS, M.D , Minneapolis, Mm.

From the routine examinations of more than six thousand children and young adults, those with definite evidence of pulmonary lesions have been followed over a period of several years They are grouped according to location of lesions

Primary tuberculous foci and their relation to the adult form of tuberculosis have been studied

Different steps in the healing of lesions are presented to show how well pulmonary lesions in childhood often heal. In another group, different steps in the advancement of pulmonary lesions are presented More than a hundred cases of pulmonary lesions among teen age girls and boys are included

Studies of Pulmonary Gangrene By I PILOT, M D (by invitation), Chicago, Ill

The purpose of these studies is to clarify a clinical condition in which the etiology has not been well understood Bacteriologic studies in our own cases reveal in most instances fusiform bacilli, spirochetes always with other bacteria particularly the pyogenic cocci As these organisms appear to be identical with those found in the oral cavity it is evident that the infection is due to invasion of mouth organisms into the lower respiratory tract For the development of lesions in the patient as well as in the experimental animal certain contributing factors are necessary In patients aspiration of mouth secretions during anesthesia and foreign bodies are outstanding predisposing causes In the rabbit material introduced intratracheally as a rule results in no change unless a foreign substance (lipiodal) is added. The Levaditi preparations of the lesions often reveal a

and chronic diarrhea were the dominant symptoms   In certain instances the emaciation amounted to a loss of 50 per cent of the usual body weight   The anemia resembled Addisonian anemia but differed in that there were no signs of postero-lateral sclerosis or of an acidity   Subjective symptoms consisting of tingling and numbness of the extremities were due to tetany and disappeared immediately with restoration of a normal blood calcium   The emaciation and anemia appear to be due to malabsorption of food   Starches and fats are poorly utilized   A high protein diet was well tolerated and did not aggravate the tetany A markedly dilated and redundant colon was present in all cases   Dietetic control and restoration of normal mineral balance resulted in a cure in two instances and improvement in the remainder

Chemical Studies of Patients with Non-Tropical Sprue   By PAUL STARR, M D , Chicago, Ill

This report consists of (1) a detailed account of the results of diet, thyroid, parathyroid and calcium medication on the metabolic rate, weight, blood calcium and stools of a patient with non-tropical sprue observed from May 1926 to November 1928   (2) the chemical data of calcium balance metabolism experiments on a second patient on low fat, high protein diet, normal high fat diet, and normal low fat diet, covering twenty-four three day periods during five months observations, (3) the effect of gastric digestion on the blood calcium in normal patients with a few observations on patients with sprue, consisting of a tabulation of the change in the blood calcium during a fractional meal in twenty instances   The suggestions in regard to the calcium metabolism of sprue to be drawn from these studies are as follows   (1) Chronic latent tetany is present in non-tropical sprue   (2) it may become acute with extreme depression of the blood calcium, (3) negative calcium balance could not be detected on low fat diets, (4) negative calcium balance was found on a high fat diet during diarrhea, (5) treatment calculated to correct this is beneficial, (6) there is an associated depressed basal metabolic rate, and (7) benefit from raising it by thyroid administration, (8) there is a slight but definite depression of the blood calcium during the gastric digestion of a fractional test-meal, (9) this is not dependent upon acid secretion

Hyperparathyroidism   By HAROLD BULGER, M D , HENRY H DIXON, M D (by invitation) and DAVID P BARR, M D , St Louis, Mo

We have studied a patient presenting multiple giant-cell bone tumors, hypotonicity of muscles, bilateral nephrolithiasis, hypercalcemia and abnormal tendency to excrete calcium in the urine and a parathyroid tumor

The removal of the parathyroid tumor caused severe and almost fatal tetany, hypocalcemia, change in the size of the bone tumors and abnormal tendency to the retention of calcium

The symptoms of this patient closely resemble many cases which are scattered

The Veins of the Suprarenal Glands in Patients with Normal and High Blood Pressure A Study of the Musculature and Volume in 100 Cases By EDGAR V ALLEN, M.D (by invitation) Rochester, Minn

The suprarenal glands removed at autopsy from 50 cases each with normal and high blood pressure were studied with particular reference to the musculature of the veins and the venous vascularization About 1400 veins were so studied by sectioning the adrenals at areas 1 cm apart, staining with Van Gieson connective tissue stain and projecting at magnifications varying from 50 to 200 times The areas of the lumens of the veins were then calculated by means of a planimeter and the readings corrected statistically

The results of this study determine the condition of the musculature of the suprarenal veins in the two clinical conditions and give evidence concerning the amount of vascularization by veins The relation of the condition of the musculature and clinical hypertension is discussed

Repeated Glucose Tolerance Tests By HENRY J JOHN, M D , Cleveland, Ohio

The study of borderline cases of diabetes through repeated glucose tolerance tests over a period of years offers interesting observations as to the evolution or the "keeping in check" of diabetes in patients under different living conditions It also gives us a definite guide as to the degree of improvement in the patient's condition and thus tells us the degree of liberty we can take with his diet Or again it shows a retrogression, due to carelessness in diet infections mental strains or inadequate treatment. Briefly it opens up a study, which after a sufficient length of time ought to throw some light on the fluctuations of the carbohydrate metabolism under varied conditions and what I consider most important—it ought to tell us just what the status of the borderline group of cases is and through that give us a definite idea as to what is the best way of dealing with this group—in other words it will lead us into preventive medicine in the field of diabetes

Hyperinsulinism By FRANK N ALLAN M.D (by invitation), Rochester, Minn

Hyperinsulinism is now recognized as a clinical entity, as a result of the study of a case with a constant tendency to hypoglycemia, previously reported in collaboration with Wilder, Power and Robertson In this case the cause of the trouble was shown to be overproduction of insulin from a carcinoma originating in the islands of the pancreas Recently two cases have been under observation, presenting a similar tendency to hypoglycemia, necessitating the ingestion of food or sugar at regular intervals during the day and night to prevent the occurrence of mental confusion, unconsciousness, and convulsions Hyperinsulinism is suspected, although it cannot be definitely proven In one case the pancreas appeared normal on surgical exploration, and microscopic examination failed to reveal any abnormality Removal of a part of the pancreas has diminished but has not completely abolished the tendency to hypoglycemia. The patient no longer requires feeding at night and can remain free of symptoms with only three meals during the day

characteristic distribution of the fusospirochetes Neosalvarsan in certain cases if given early has a favorable influence but in most instances of the diffuse types of gangrene the outcome is unfavorable

Studies in Syphilis of the Stomach By HARRY A SINGER, M D (by invitation), Chicago, Ill

According to current medical literature, there is a marked discrepancy between the incidence of syphilis of the stomach in the morgue as compared with its occurrence in the clinic The chief reason for this difference is variance in opinion as to what constitutes from the anatomic standpoint gastric lues Pathologists are inclined to disregard all but those cases in which specific changes are demonstrable A study of a series of cases from the combined clinical and morphological standpoints indicate that at the time most patients with syphilis of the stomach reach the operating or post-mortem table, the lesions are receding or healing and therefore from the microscopic viewpoint not typical of lues A gradual transition from the active lesion with its classical luetic picture to the healing and healed lesion can be traced by comparing the histologic slides from a series of resected stomachs

The Course of Syphilitic Heart Disease By FREDRICK A WILLIUS, M D , Rochester, Mln

This study was undertaken to attempt to determine the course of and the factors influencing the expectation of life in patients suffering from cardiovascular syphilis

From a large material it was possible to select only one hundred cases in which a definite history of the onset of syphilis was available, and in which the date and cause of death could be accurately determined

These cases were divided into three groups the first group comprising those cases presenting clinical evidence of syphilitic aortitis, there were seventeen cases, all males The second group presenting clinical evidence of syphilitic aortitis associated with aortic insufficiency, comprised fifty-nine males The third group comprised cases of thoracic aneurism, of which there were thirty-four, all being males

The groups are considered separately but in the abstract it seems desirable to present average statistics only

The average age at which syphilis was contracted was twenty-seven years and the average age at which death occurred from heart disease was fifty-seven years, in other words, thirty years elapsed from the time that the disease was contracted until the patient died of heart disease

Numerous other points are discussed, viz the incidence of positive serology of the blood and of the spinal fluid in the various groups, the incidence of associated syphilitic manifestations, the types of cardiac manifestations and the occurrence of associated electrocardiographic phenomena

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Determination of Glucose in Tissues from Normal Hyper- and Hypoglycemia, and
    Depancreatized Animals   By WILLIAM THALHIMER, M D , and MARGARET C
    PERRY, A M (by invitation), Milwaukee, Wis
    The method used for determining glucose in tissue is new   The accuracy of
the method was tested by many duplicate determinations, by the recovery of a
known amount of glucose added to tissue at various steps of the procedure, and
in other ways   Analyses were made which seemed to give accurate determina-
tions of "true" and "apparent" glucose in tissues   Very little variation in the
amount of "true" glucose in tissues was found in the different conditions studied

Blood Urobilin   By M A BLANKENHORN, M D , Cleveland, Ohio
    Normal urobilin content of human blood has not been described   Urobilin
is frequently found in the blood under certain pathological conditions in large
amounts, and is a constant finding in small amounts in normal urine   It has
been considered a threshold substance   By refinement in technique of the
Schlesinger fluorescence test, human blood can be shown to have a fairly constant
amount of urobilin   The method is described, and the amount as determined
from one hundred and twenty-eight normals is reported

The Untoward Effects of Phenylhydrazin in Certain Cases of Polycythemia Vera
    H Z GIFFIN, M D , and H M CONNER, M D (by invitation), Rochester,
    Minn
    The histories of three patients are presented, in which the use of phenylhydrazin
was followed by fatal outcome   In view of the excellent therapeutic results that
have been reported in polycythemia vera following the use phenylhydrazin it
seems wise to utter this word of caution with respect to its use   Analysis is made
of the characteristics of the cases in which the drug should be used with great
caution, if at all

Serum Complement Fixation Tests in Blastomycosis and in Monilia Infections
    By O GARCIA, M D (by invitation), St Louis, Mo
    Agglutination tests for blastomycosis have already been described by a number
of workers   A survey of the literature reveals no successful work on complement
fixation in blastomycosis
    In a series of experiments both on blastomycosis and monilia infections, it has
been possible to develop antigens which can be used for complement fixation tests
    Positive tests have been secured on clinical cases of blastomycosis and also on
monilia infections of the mouth, lungs and gastrointestinal tract
    Animals receiving experimental inoculations with these fungi develop definite
complement fixing antibodies

The Intradermal Salt Test in Fever   By CHARLES DUDEN, M D (by invitation)
    and DAVID P BARR, M D , St Louis, Mo
    The intradermal salt test has been applied to the study of fever   The disap-

pearance time of the intradermally injected salt is regularly diminished in bacterial fevers and in fevers caused by foreign protein injection   It is unchanged in fevers of equal degree produced by external heat

Systemic Histamine-like Reactions in Allergy Due to Cold   A Report of Three Cases   By BAYARD T HORTEN, M.D (by invitation) and GEORGE E BROWN, M.D , Rochester, Minn

This report is based on the studies carried out on three subjects, all exhibiting skin disturbances and systemic reactions following exposure to moderate grades of cold   The reactions were so similar in these cases as to constitute a most striking clinical syndrome, which, to our knowledge, has never been reported

Three cases are reported exhibiting local and general symptoms of cold allergy The local effects on the skin were Pallor during the exposure period followed by redness, swelling, increased local heat of the hands after exposure to cold Following a latency period of three to four minutes, a characteristic systemic reaction appeared, which was quite characteristic of that produced by histamine There was a fall in blood pressure, a sharp rise in pulse rate, a tendency to syncope, with transitory recovery in fifteen to thirty minutes   Complete recovery from the local reaction occurred in twelve to twenty four hours   The experimental work carried out gave evidence of the chemical nature of these reactions, and strongly suggested the release of a histamine like substance in the skin following exposure to cold which, when carried in the general circulation, produced a reaction characteristic of histamine   These clinical observations are further confirmation of the work of Lewis and associates on the presence of histamine or allied substances in the human skin

The Relation of the Weather to the Pain of Arthritis   By E B RENTSCHLER, M.D (by invitation), FRANCES VANZANT, M.D (by invitation) and L G ROWNTREE, M D , Rochester, Minn.

It has been found that in an arthritic medical service, the pain of infectious arthritis varies markedly with the changes in the weather   The condition of each patient on the service has been reported every day for a year   On completing rounds the doctors on the arthritic service have made a summary of the statements of the patients and recorded the results   At the same time records have been kept in the laboratory of barometric pressure and temperature inside and outside, humidity, sunshine, the presence or absence of storms and the electrical state

A remarkable correlation has been found between the pain of arthritis and the presence of a storm   In fact, there were only two or three storms in the entire year not reflected in the pain of the patients with arthritis   There is also a remarkable correlation between the changes in barometric pressure and the pain of arthritis   A positive correlation in more than 70 per cent and some sort of correlation in more than 90 per cent of cases has been observed   The amount of

sunshine also exercises a marked influence   Certain patients claim a capacity to predict storms by their pain and have been checked, and one has been found to be correct over 95 per cent of the time   In patients who believed that the weather in no way influenced their pain almost as striking a correlation was found at times   This study brings up many considerations which have largely been overlooked by the physician

Urinary Proteins   By WILLIAM A THOMAS, M D (by invitation), Chicago, Ill
In the production of artificial uremia in dogs, there is obtained a protein in the urine which does not originate in the blood   This protein has been identified by appropriate precipitation tests as coming from viscera and muscle and is definite in its immunological reactions

Later, this disappears and is replaced by serum protein in large quantities   Demonstration of this protein from several organs has been successful

Later the process so far breaks down this protein that it is not recognizable as such, but combines with serum protein and is excreted in this combination

The mechanism of excretion of protein through the kidney damaged or undamaged is discussed and it is believed that the kidney is not permeable to protein which belongs in the circulation of that species but is excreted only when altered by chemical, osmotic or other factors to such an extent that it is no longer the normal serum protein of that organism and that the liver protein combines with serum protein as a mechanism of detoxication and excretion

Symptomatic Relapses During Liver-Induced Remissions in Pernicious Anemia
By CYRUS C STRUGIS, M D , and RAPHAEL ISAACS, M D (by invitation), Ann Arbor, Mich
Many patients with pernicious anemia whose blood has returned to normal while taking liver or an adequate liver fraction, experience, during the months following the development of the remission, certain symptoms which are similar to those of a beginning relapse   These include nausea, loss of appetite, weakness, nervousness, diarrhea or constipation, increase in "numbness" of hands and feet, sore tongue and ease of fatigue   If the liver or liver extract is continued until these symptoms pass away or decrease, a true "hematological" relapse is prevented   Some patients discontinue the liver at this time and the anemia reappears   It is evident that the active liver component acts by supplying a substance, the power to elaborate which has been lost, and is never regained   The treatment is symptomatic and does not influence the fundamental disease process   The periodicity of the relapses and remissions remains a characteristic, even though the blood (and bone marrow) have been restored to normal function

COMPOSITION OF THE DIETS EMPLOYED

Diet A

Substance	Weight	Protein	Fat	Carbo-hydrate	Calories	Ca	P
	grams	grams	grams	grams		grams	grams
Egg	100	13 4	10 5		148 0	0 067	0 180
Bread	30	2 8	0 4	15 8	77 7	0 008	0 028
Butter	10	0 1	8 5		76 9	0 002	0 002
Lettuce	30	0 4	0 1	0 9	5 7	0 013	0 013
Fresh tomato	50	0 5	0 2	2 0	11 5	0 006	0 013
Sugar	10			10 0	40 0		
Egg	20	2 7	2 1		29 6	0 013	0 036
Cream (18 per cent)	100	2 5	18 5	4 5	195 0	0 086	0 067
Total		22 4	40 3	33 2	584 4	0 195	0 339

ERRATUM

When binding this volume insert the attached tables in Volume VII, Number 1 after page 96

Diet C

Substance	Weight	Protein	Fat	Carbo-hydrate	Calories	Ca	P
	grams	grams	grams	grams		grams	grams
Egg	50	6 7	5 3		74 0	0 034	0 090
Egg yolk	100	15 7	33 3		363 0	0 137	0 567
Cream (18 per cent)	50	1 3	9 3	2 3	77 5	0 073	0 017
Sugar	10			10 0	40 0		
Apple	100	0 4	0 5	14 2	63 0	0 007	0 012
Egg white	30	3 7	0 1		15 3	0 005	0 004
Shredded Wheat	35	4 2	0 6	26 3	127 8	0 014	0 103
Milk	200	6 6	8 0	10 0	138 0	0 240	0 186
Sugar	10			10 0	80 0		
Total.		38 6	57 1	72 8	958 6	0 480	0 939

Diet D

Substance	Weight	Protein	Fat	Carbo-hydrate	Calories	Ca	P
	grams	grams	grams	grams		grams	grams
Egg (whole)	170	25 8	17 9		251 6	0 1139	0 3060
Egg (white)	30	3 7	0 1		15 3	0 0045	0 0050
Egg (yolk)	100	15 7	33 3		363 0	0 1370	0 5240
Cream (18 per cent)	110	2 8	20 4	5 0	214 5	0 0946	0 0737
Sugar	30			30 0	120 0		
Apple	100	0 4	0 5	14 2	63 0	0 0070	0 0120
Milk	700	23 1	28 0	35 0	483 0	0 8400	0 6510
Shredded Wheat	35	4 2	0 6	26 3	127 8	0 0144	0 1134
Butter	50	0 5	42 5		384 5	0 0045	0 0085
Tenderloin	100	16 2	24 4		284 0	0 0093	0 1725
Lettuce	40	0 5	0 1	1 2	7 6	0 0172	0 0168
Asparagus	100	1 8	0 2	3 3	22 0	0 0250	0 0390
Bread	80	7 4	1 0	42 2	207 2	0 0216	0 0744
Celery	40	0 4		1 3	7 6	0 0312	0 0148
Cauliflower	100	1 8	0 5	4 7	31 0	0 1230	0 0610
Banana	100	1 3	0 6	22 0	99 0	0 0090	0 0310
Total		102 6	170 1	185 2	2,681 1	1 45	2 1023
Analyzed						0 994	1 756

Diet L

Substance	Weight	Protein	Fat	Carbo hydrate	Calories	Ca	P
	grams	grams	grams	grams		grams	grams
Apple	330	1 3	1 7	46 9	207 9	0 0231	0 0396
Sugar	39			39 0	156 0		
Egg white	120	14 8	0 2		61 2	0 0180	0 0168
Cream (40 per cent)	195	4 3	78 0	5 9	743 0	0 1677	0 1307
Bread	195	18 7	2 7	99 6	498 3	0 0527	0 1814
Butter	75	0 8	63 8		576 8	0 0113	0 0128
Total		39 9	146 4	191 4	2,243 2	0 273	0 381
Analyzed						0 218	0 345

STUDIES OF SERUM ELECTROLYTES

IV The Chloride and Nitrogen Balances, and Weight Changes in Pneumonia[1]

By F WILLIAM SUNDERMAN[2]

(From the John Herr Musser Department of Research Medicine, University of Pennsylvania and the Medical Wards[3] of the Pennsylvania Hospital Philadelphia)

In an earlier study (1) data were presented on the changes in the blood serum during the course of lobar penumonia It was found that the concentration of total base, chloride, and protein in the serum was usually decreased during the active infection Following the crisis these concentrations returned to their normal values, the chloride less rapidly than the total base The low excretion of chloride in the urine during the precritical period has long been known and the inference has been drawn that there is a correlation between the urinary excretion and the serum concentration No satisfactory explanation of the mechanism of the change in composition of the serum or of the decreased urinary excretion of chloride has been established, but from studies in the literature it has often been inferred that during the precritical period the body tissues retain chloride which is subsequently released during the epicritical period In an attempt to obtain data on factors concerned in these changes, studies of the intake and output were undertaken in patients suffering with lobar pneumonia

To review completely the very extensive literature upon the chloride concentration of the serum and urine in pneumonia seems unnecessary since this has been done by many authors, notably by Hutchison (6), Von Moraczewski (8),

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[2] Robert M Girvin Fellow in Research Medicine.

[3] Service of Dr George W Norris

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Garratt (7), and Peabody (9)   The conclusion of the earlier workers that chloride was retained in the body during the febrile period arose chiefly from the observation that the addition of chloride to the diet during this period was not accompanied by a corresponding increase of chloride excretion   These observations were later supplemented by analyses of tissues and studies of chloride balance Von Moraczewski (8) reported 18 cases of pneumonia in which daily amounts of chloride, nitrogen, phosphorus, and calcium in the food, urine, and feces were determined   Only three times in his series, twice when the food intake was very small and once when calcium phosphate was administered, did he find the daily excretion of chloride in the urine and feces in excess of the daily intake of chloride He observed that although the excretion of chloride in pneumonia parallels in general the intake of chloride, nevertheless, an excessive ingestion of salt was not accompanied by the normally prompt excretion

Hutchison (6) reported 4 cases that excreted less chloride in the urine during the febrile period than was taken with the food, but these patients received from 1 0 to 7 5 grams of chloride per day   Hutchison found no evidence from tissue analyses that chloride tended to accumulate in any particular organ during pneumonia, but rather that all of the organs seemed to contain somewhat more chloride per unit of weight than normally

In order to determine how much chloride could be retained in the pneumonic exudate, Peabody (9) analyzed the lungs in two cases of pneumonia in which one lung was normal and the other hepatized   He found that a retention of about two grams of sodium chloride could be credited to the pneumonic lung   The skin of pneumonia patients showed no significant increase in its salt content   Von Hosslin (10) averaged the percentile concentration of chloride in normal and pneumonic lungs from his own data and from data of Jarisch (11), Meillère (12), Hutchison (6), and Terray (13) and found that the mean sodium chloride concentration in pneumonic lungs in 14 observations was 0 343 per cent of fresh weight and in 10 estimations in normal lungs, 0 331 per cent   Taking the weight of a normal lung at 250 grams and a hepatized lung at 1250 grams, there would be an increase of about 3 5 grams of sodium chloride in the latter

Sandelowsky (2) gave from 3 to 36 grams of sodium chloride in 3 days to patients convalescing from pneumonia   He used himself as a control and observed in the pneumonia patients a greater delay in the excretion of the sodium chloride

Prigge (14) upon injecting from 100 to 150 cc. of a 25 per cent solution of sodium chloride intravenously into patients during the precritical period of lobar pneumonia also observed a lagging in the excretion of the chloride, although the blood concentration fell the day following injection and thus behaved as in normal individuals

Holten (15) fed pneumonia patients during the precritical period a diet which contained between 6 and 7 grams of sodium chloride daily and found that less than the corresponding amount of chloride was excreted in the urine until about the time of crisis

Leyden (5) in 1869 was perhaps the first to study weight changes in pneumonia. He observed in his patients an increased loss of weight through the skin and lungs during the febrile period and a rapid loss following the crisis. Sandelowsky (2) studied the body weights in 11 cases of pneumonia. In 7 cases the body weight either increased slightly during the precritical period or remained constant, where as, after the crisis the weight fell more or less rapidly, in 4 there was a continued decrease in weight during the febrile and afebrile periods

Garner and Sabareanu (3) observed that pneumonia patients do not always lose weight in the early stages of the disease but that even with a small food intake they sometimes gain weight. The gain in weight in their cases did not generally exceed 500 grams per day but was in one instance as high as 2000 grams in three days With fall in temperature the weight dropped In children suffering with pneumonia the gain in weight before the crisis would appear to occur more fre quently than in adults Lussky and Friedstein (4) observed in their series of children that the weight remained constant or was increased before crisis, but was decreased with the disappearance of fever Only one out of their 28 cases occur ring in children failed to show loss of weight following the crisis

MATERIAL AND METHODS

These studies were carried out on seven patients with lobar pneumonia In five patients study was commenced between the fifth and eighth days of the disease Two patients, selected because they were admitted on the third day of the disease, were treated with large doses of sodium chloride by mouth The period of investi gation for each patient was from 2 to 11 days and except in one fatal case extended 1 to 8 days following the crisis The patients selected were young adult males who had all been healthy before the onset of pneumonia. In addition two normal individuals were followed by similar methods of study for 48 hours on a diet com parable with that received by the patients. The normal individuals were sub jected each day to a cabinet light bath designed to promote a loss of water through the skin which would approach in magnitude that occurring in the patients

On admission the patients were placed in a special metabolic room and were under constant observation during the period of study one patient or occasionally two being studied at a time Each morning at 7 o'clock the patient was weighed A silk scale of the type proposed by Benedict and Root (16), which weighs accu rately to 10 grams, was used and was fitted with an especially constructed thin stretcher With the exception of the first 2 days of study in Case B3, no patient received any food for at least 8 hours before weighing time, and none received water or food between 5.30 and 7 o'clock in the morning The patient was en couraged to defecate and void during the half hour before weighing If unable to void, he was catheterized

The daily intake consisted of weighed amounts of the following ingredients milk, grape juice, tea, canned tomato and pea purees, triscuit, sugar, salt and water The diet corresponded to the type given to the hospital patients suffering

from lobar pneumonia but was designed to contain articles which have an approximately uniform chemical analysis   Analyses of the commercial foods were furnished to us by the respective manufacturers, these were supplemented by our own analyses and by values taken from the Food Bulletin of the Connecticut Agricultural Experiment Station (17)   The chloride content of the foods was analyzed

In one patient (B3) a high caloric diet consisting of cream, milk, eggs, and malted milk was given for 2 days

Enemas that were given contained small amounts of soap

The collected measured output included urine, feces, sputum, and blood removed for analysis   These were weighed to a gram   Toluol was used to preserve the urine   The excreta were kept refrigerated   An attempt to collect the sweat was unsatisfactory

The dry weights of excreta and the nitrogen contents of the urine and feces were determined   The total amount of nitrogen excreted per day in the sputum was found in two analyses to be negligibly small   The chlorides were analyzed according to Van Slyke's method (18) in serum and food and Volhard's in urine   The Kjehldahl method was used for nitrogen determination

RESULTS

In table 1 are given data on the food intake, nitrogen and chloride balance and changes of weight   The day of crisis is designated as the zero day and days before and after as − and + respectively   An estimate of the caloric requirement has been made from normal basal standards for weight and age with an addition for fever, estimated by the average percentile increase of metabolism per degree increase of temperature from Du Bois' data (19) and an added 10 per cent during the precritical period   The caloric value of the diets was consistently low and the nitrogen balance markedly negative   An attempt to increase the intake of food in patient B3 led to serious tympanites and for this series of studies such attempts were not repeated   Patients B6 and B7, who were given large doses of sodium chloride, took more food spontaneously during the precritical period without deleterious symptoms

Weight changes

Loss of weight was usually present in both precritical and postcritical periods as would be expected with such an inadequate diet   In table 2 is shown the relative daily loss of weight in grams per kilogram of initial weight and the negative nitrogen balance per kilogram

of initial weight for the patients studied and, for comparison, similar data from a series of fasting experiments from Benedict's (20) collection of studies   The weight curve during the postcritical period of B5 was possibly modified by the development of a pleural effusion B6 and B7 received large amounts of NaCl which in B7 were poorly excreted and led eventually to slight visible edema   Patients B3 and B4 exhibited increased loss of weight following the crisis associated with unchanged or with less negative nitrogen balance and less caloric deficit   A similar increased loss of weight following the crisis was noted by Leyden (5) and interpreted by him to indicate precritical storage of water and postcritical release   The behavior of the weight curves resembles that observed in fasting studies   The fluctuations in the rate of loss of weight from day to day can be approximately duplicated in fasting studies   Partial inanition is evidently an important factor in inducing the observed changes in weight with apparently additional factors diminishing the rate of loss of weight before the crisis, and increasing it after the crisis

That nitrogen equilibrium is not maintained in pneumonia has been demonstrated by many workers, including von Moraczewski (8), Cook (21), and Leyden and Klemperer (22)   The negative nitrogen balance in the pneumonia patients was higher than in fasting subjects doubtless because of the greater metabolism in the former   The loss of weight per milligram of negative nitrogen balance was less in the precritical periods in all the patients and throughout the study of B5 and in the two subjects receiving salt than in the fasting subjects This might be considered indirect evidence of relative retention of water during the precritical period   In the postcritical periods of B3 and B4 the loss of weight was closely comparable with that observed in the fasting subjects

Excretion through skin and lungs

In table 1 are given the data on the urinary loss of water and the loss of weight through skin and lungs. The loss of water through skin and lungs is equal to the total loss of weight through skin and lungs minus the difference between weight of CO_2 given off and of O_2 absorbed through the lungs   This difference of weight is about 75 ±75 grams per day   It is evident from these data that from 25

TABLE 1
Data on cases

Case number	Day from crisis	Temperature (°F)	Initial weight and daily change (gm)	Urinary water (gm)	Weight loss, skin and lungs (gm)	Intake: Protein (gm)	Intake: Carbohydrate (gm)	Intake: Fat (gm)	Intake: Calories	Estimated caloric deficit	Nitrogen output (gm)	Nitrogen balance (gm)	Chloride intake (mM)	Chloride output: Urine (mM)	Chloride output: Feces (mM)	Chloride output: Sputum (mM)	Chloride output: In blood removed for analysis (mM)	Chloride output: Total (mM)	Chloride balance (mM)	Expected chloride balance = 50 per cent (mM)	Relative chloride balance	Amount of sputum (gm)
B1	−1	103	50,166 / −350	1,002	3,040	25	77	38	755	1,400	17	−13	17	0.2	2.5	5.1	3.0	20 ±9	−3 ±9	−13	?	88
	0	104	−306	1,021	2,744	26	102	31	799	1,300	20	−16	17	0.0	1.3	11.6	0.6	25 ±9	−8 ±9	−11	?	152
	+1	98	−1,412	999	2,711	30	87	35	786	800	22	−17	21	0.7		5.8		17 ±9	+1 ±9	+49	+	86
B2	−3	105	48,138 / +149	1,342	2,196	38	112	46	1,067	800	23	−17	36	36.9		2.8	4.2	53 ±9	−17 ±10	+5	−	35
	−2	105																				
B3	−3	104	86,138 / +186	1,473	2,436	86	215	153	2,733	900	26	−12	137	18.7	2.4	8.1	4.0	40 ±9	+97 ±12	+7	+	108
	−2	104	−640	1,494	2,174	46	118	74	1,412	2,200	27	−20	89	10.8		3.7	5.3	31 ±9	+58 ±11	−22	+	57
	−1	103	−1,260	1,616	2,197	0	8	0	333	3,200	26	−26	0	4.1		3.8		17 ±9	−17 ±9	−44	?	50
	0	105	−1,106	1,327	4,276	12	52	14	391	3,100	23	−21	7	2.9	0.3	2.8		15 ±9	−8 ±9	−39	+	43
	+1	100	+610	1,813	2,584	41	141	47	1,160	1,600	36	−29	27	3.3		1.6	3.8	18 ±9	+9 ±10	−21	+	12
	+2	100	+623	2,067	2,080	52	110	63	1,227	1,600	27	−19	39	11.4	1.7	2.6		23 ±9	+16 ±10	−22	+	31
	+3	100	+1,324	2,964	2,382	48	100	57	1,117	1,700	31	−23	111	57.3		1.8	3.8	74 ±9	+37 ±11	−46	+	20
	+4	98	+1,141	2,715	2,274	64	143	74	1,500	800	26	−16	98	76.3	1.4	3.4		90 ±9	+7 ±11	−40	+	40
	+5	98	+554	2,306	1,829	33	132	36	1,002	1,300	23	−18	125	155.0		0.5	3.3	165 ±9	−40 ±12	−19	?	6
	+6	98	+1,559	2,259	1,997	35	148	35	1,060	1,200	20	−14	74	164.0	1.5			178 ±9	−104 ±10	−54	−	0
	+7	98	−639	1,919	1,690	11	100	9	555	1,700	17	−15	86	134.0				143 ±9	−58 ±11	−22	−	0

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B4				60,934																			
	−2	104	+	149	1,419	3,223	30	87	37	800	1 900	32	−27	22	39 5			5 0	54 ±9	−32 ± 9	+5	−	0
	−1	103	+	149	1,716	3,223	35	104	42	939	1,700	32	−26	25	31 6	0 6	1 8	1 8	45 ±9	−20 ±10	+5	−	60
	0	102	−	2,020	2,983	3 086	34	90	40	861	1 600	32	−27	25	37 6		1 0	3 6	51 ±9	−26 ±10	−71	+	26
	+1	99	−	1,950	1,667	2 554	28	93	34	798	1 400	26	−22	20	21 0	3 6		2 8	36 ±9	−17 ± 9	−68	+	0
	+2	99	−	1,013	1,443	1,909	27	75	33	698	1,500	22	−18	20	17 7				27 ±9	−7 ± 9	−35	+	8
	+3	99	+	156	1 716	1,689	34	107	41	936	1,300	24	−19	24	16 7	1 9	0 3	1 9	30 ±9	−6 ± 9	+5	?	7
	+4	98	−	1,086	3,031	1,631	35	124	41	1 014	800	17	−11	24	29 0				38 ±9	−14 ± 9	−38	?	3
	+5	98	−	582	2 178	1 499	33	122	35	943	900	18	−13	56	21 7			3 3	34 ±9	+22 ±10	−20	+	5
	+6	98	+	283	1,569	2 029	49	212	48	1,508	300	17	−14	143	48 9		0 7		59 ±9	+84 ±12	+10	+	11
	+7	98	+	276	3,360	717	100	188	172	2 707		21	−5	152	164 0	0 9		2 2	176 ±9	−24 ±12	+10	−	0
	+8	98	−	794	2 147	927	67	155	113	1,924		23	−13	121	171 0	2 1			182 ±9	−61 ±11	−28	−	0
B5				26 528																			
	−3	103	−	198	1,520	1 774	35	92	42	890	600	13	−7	26	22 9	2 1	0 1	2 0	36 ±9	−11 ±10	−7	?	24
	−2	104	−	284	2,405	924	30	83	36	773	800	14	−9	22	30 2	0 4	2 9		43 ±9	−21 ± 9	−10	?	36
	−1	103	−	56	1,679	813	27	94	32	782	700	11	−7	19	13 6		4 6	3 1	30 ±9	−11 ± 9	−2	?	47
	0	103	−	731	2,340	896	27	108	32	840	700	14	−10	19	13 2	3 0	3 8		29 ±9	−11 ± 9	−26	?	45
	+1	100	−	389	1,922	941	24	92	26	707	400	9	−5	43	4 1	0 9	2 4	3 3	20 ±9	+23 ±10	−14	+	28
	+2	100	+	730	1,601	649	46	187	45	1,361		8	−1	94	4 9		1 4		15 ±9	+78 ±11	+26	+	19
	+3	100	+	398	961	825	56	111	98	1,557		11	−2	132	9 4	3 4	0 1		22 ±9	+110 ±12	+14	+	10
B6				50,625																			
	−5	102	−	468	1,788	2,516	40	116	48	1,063	900	18	−12	137	17 3		4 6	3 5	34 ±9	+103 ±12	−16	+	48
	−1	101	+	751	1,420	2 085	53	130	64	1,303	500	21	−16	211	37 0	1 5	14 2		62 ±9	+149 ±13	+26	+	97
	−3	101	+	78	2,140	1,970	55	137	63	1,340	500	15	−6	296	112 0	0 6	10 4	5 5	138 ±9	+158 ±15	+3	+	86
	−2	101	−	545	1,909	1,822	46	158	58	1,369	400	16	−13	291	231 0	1 3	9 5	4 2	255 ±9	+36 ±15	−19	+	64
	−1	101	−	64	1,941	1,856	49	148	59	1 340	500	14	−6	527	346 0	1 0	14 2		370 ±9	+157 ±20	−2	+	119
	0	101	−	671	2,342	1,758	45	157	51	1 299	500	12	−5	344	316 0		6 3	4 3	336 ±9	+8 ±16	−24	+	60
	+1	100	−	333	1,915	1 815	50	138	57	1,270	400	13	−5	332	288 0	0 3	6 5		304 ±9	+28 ±16	−12	+	54
	+2	100	−	609	1,716	2,061	51	152	60	1,370	300	13	−5	315	292 0	0 3	6 5	4 3	312 ±9	+3 ±15	−21	?	49
	+3	100	−	879	1,577	1,822	48	127	51	1,187	500	14	−6	224	295 0	0 6	7 7		312 ±9	−88 ±13	−31	−	52
	+4	100	−	752	1,196	1,878	45	105	53	1,090	600	12	−5	351	192 0	0 2	3 4		205 ±9	+146 ±16	−26	+	24

TABLE 1—*Concluded*

Case number	Day from crisis	Temperature* (°F)	Initial weight and daily change (gm)	Urinary water (gm)	Weight loss skin and lungs (gm)	Intake: Protein (gm)	Carbohydrate (gm)	Fat (gm)	Calories	Estimated caloric deficit	Nitrogen output (gm)	Nitrogen balance (gm)	Chloride intake (mM)	Chloride output: Urine (mM)	Feces (mM)	Sputum (mM)	In blood removed for analysis (mM)	Total (mM)	Chloride balance (mM)	Expected chloride balance =50 per cent (mM)	Relative chloride balance	Amount of sputum (gm)
B7	−6	104	59,278 / 0	1,107	1,703	39	113	46	1,030	1,400	10	−4	96	2.0	6.3	15.1	3.8	30 ±9	+66 ±11	0	+	119
	−5	102	+71	1,090	2,113	44	97	53	1,050	1,100	12	−5	203	2.9		11.2		29 ±9	+174 ±13	0	+	90
	−4	100	+914	2,115	1,278	52	108	63	1,207	900	25	−17	296	24.8	6.9	10.9	4.1	49 ±9	+247 ±15	+32	+	80
	−3	100	+129	1,932	1,658	46	109	57	1,137	1,000	19	−12	291	71.5		12.7		104 ±9	+187 ±15	+5	+	113
	−2	100	+1,211	1,952	795	42	122	51	1,137	1,000	12	−5	530	116.6	1.8	13.1	4.1	139 ±9	+391 ±20	+42	+	100
	−1	100	+1,602	1,293	989	42	122	51	1,137	1,000	18	−11	344	83.4	1.4	13.8		113 ±9	+232 ±16	+56	+	106
	0	99	+1,715	983	872	47	103	57	1,117	900	10	−2	239	47.0	0.9	9.4	4.5	67 ±9	+172 ±11	+60	+	72
	+1	99	+340	1,004	1,018	29	87	35	790	1,200	9	−4	41	38.4		12.5		65 ±9	−24 ±10	+12	−	83
	+2	98	−354	1,022	802	20	71	23	595	1,300	8	−5	34	39.5	0.9	10.2	4.4	60 ±9	−26 ±10	−12	?	78
	+3	98	−711	1,097	868	36	92	43	910	1,000	9	−3	69	40.9	0.5	10.6		61 ±9	+8 ±10	−2	?	76
Normal subjects B8	1		68,046 / −865	1,336	2,502	37	81	40	830	1,500	13	−10	34									
	2		−482	1,059	2,092	39	85	41	850	1,400	13	−7	34									
B9	1		74,184 / −1,411	2,703	2,079	36	70	38	765	1,700	11	−5	17									
	2		−843	1,676	2,034	36	75	40	800	1,700	13	−7	34									

* Average of maximum and minimum temperatures for the day

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TABLE 2
Weight and nitrogen balance in pneumonia and in fasting subjects (from literature)

Subject	Period days	Loss of weight, grams per kilogram per day			Negative N balance mgm. per kilogram per day			Weight loss / N balance	Caloric deficit / Caloric need
		Mean for period	Maximum	Minimum	Mean for period	Maximum	Minimum		
Fasting men									
Succi	3rd to 12th	9 2	20 6	1 6	169	242	118	55	1 00
	3rd to 12th	7 9	15 9	1 6	151	214	110	52	1 00
	3rd to 12th	7 2	10 3	+1 6					1 00
	3rd to 12th	7 9	17 5	+1 6	114	154	80	69	1 00
	3rd to 12th	8 1	14 3	+4 8					1 00
	3rd to 12th	8 4	24 6	0					1 00
	3rd to 12th				129	155	88		1 00
	3rd to 12th	6 2	9 7	4 2					1 00
Jacques	3rd to 12th	7 3	30 2	+3 2					1 00
Beanté	3rd to 12th	6 7	12 3	0	157	208	127	43	1 00
Schenk	3rd to 12th	5 5	12 5	1 8	114	141	78	48	1 00
L	3rd to 12th	7 1	12 5	1 8	172	195	161	41	1 00
Pneumonia									
B3	Precritical	6 7			225			30	0 61
	Postcritical	10 8			226			48	0 62
	Total	9 8	18 1	+2 2	226	338	140	43	0 62
B4	Precritical	(+) 2 4			435			(+) 6	0 68
	Postcritical	12 3			259			47	0 37
	Total	9 6	33 2	+4 6	291	443	82	33	0 44
B5 (pleural effu sion)	Precritical	0 7			290			2	0 46
	Postcritical	0 0			170			0	0 08
	Total	0 3	2 8	+2 8	221	377	38	1	0 27
B6 (with salt)	Precritical	0 1			209			0	0 30
	Postcritical	1 2			103			12	0 27
	Total	0 7	1 7	+1 5	156	316	99	4	0 29
B7 (with salt)	Precritical	(+) 10 9			152			(+) 72	0 49
	Postcritical	(+) 6 9			59			(+) 117	0 56
	Total	(+) 9 3	6 0	+29 0	115	286	34	(+) 81	0 52

per cent to as high as 80 per cent of the total water output in these subjects is by way of the skin and lungs   This is correlated of course with the visible sweating observed through much of the period studied Such visible sweating precludes the possibility of calculating metabolism by the method of Benedict from the loss of weight through skin and lungs   Marked increase in loss of weight by skin and lungs just at the time of the crisis was not an outstanding feature of our cases The high skin-lung loss of weight in the normal subjects, B8 and B9, was the result of a daily cabinet bath

It is noteworthy that although the normal subjects B8 and B9 forced drinking of water to the point of discomfort they did not attain the level of water intake and water output reached by the pneumonia patients without any especial persuasion

Chloride balance

Our chloride balance studies may be divided into two groups consisting of four patients (B1, B2, B4 and B5) who received no unusual form of treatment, and three patients (B3, B6 and B7) two of whom were given excessive quantities of salt (B6 and B7) and the other (B3) who was given a relatively high caloric diet during the first two days of observation   The chloride in the daily intake of food, water, and enema was measured as well as the chloride output through the urine, feces, sputum, and blood removed for analysis   An allowance of ±2 per cent has been made for maximum error

The attempt was made to collect sweat for chloride analysis Strips of lint which had been washed free from chloride with distilled water were tied on to the patient's forehead, axillae, and nape of the neck   At frequent intervals the entire body was dried with other strips   However, it proved impossible to obtain satisfactory collections by this method   The best data relative to sweat in pneumonia that was found was that of Schwenkenbecker and Spitta (23)   These investigators placed patients on a rubber cloth washed free from chloride and covered them with chloride free linen   After 24 hours they washed the individual and coverings first with water, then with alcohol, and determined the chloride content of the fluid collected Their conclusion that not more than 1 gram of sodium chloride was excreted daily in the sweat in diseases with profuse sweating such as

pneumonia is consistent with the present observations, for although the collections of sweat in this study were unquestionably incomplete, the maximum daily recovery which was obtained in 19 attempts was 0 27 gram NaCl To allow for the chloride in the sweat 0 5 gram NaCl, with an allowance for error of ±0 5 gram, was arbitrarily added to the measured daily output of chloride

In table 1 are presented the individual daily chloride balances The total daily output includes the allowance for sweat The balance is designated as positive when the intake exceeds the output

The first group of our cases (B1, B2, B4 and B5) showed daily chloride loss during the precritical period Even assuming that no chloride was excreted in the sweat, the chloride balance was negative during 8 out of 10 critical and precritical days in these patients In B3, B6, and B7, on the other hand, there was a daily retention of chloride 15 out of 17 critical and precritical days The 2 days in which the balance was negative in this group occurred in B3 on the −1 and 0 day at a time when very little food was taken Case B3 had been given a relatively high caloric diet on the −3 and −2 days of his disease which had to be discontinued on the days following on account of tympanites After the crisis in the first group and in B3 there was retention of chloride during the first 4 days During the same period B7 showed a definite loss of chloride and B6, fluctuations in the balance

This evidence suggests that more chloride is excreted than ingested during the precritical period in lobar pneumonia when the patients receive no forced diet or excessive quantity of salt and that the situation is reversed when larger quantities of chloride are given

A question which arises in considering the chloride balance is its relation to gain or loss of body water Associated with a gain or loss of body weight we may properly expect a positive or negative chloride balance respectively Taking 35 mM per kilogram of body weight as the mean concentration of chloride in the body as a whole, we may approximate an expected chloride balance from a given change in weight This has been done subject to an allowance of 50 per cent for error and the increase or decrease of chloride so estimated from the change in weight has been designated in the table as the *expected chloride balance*

The difference between the chloride balance and the expected chloride balance has been indicated merely in a directional manner in the table as the *daily relative chloride balance*   The direction of this quantity has been indicated by + and − signs where the difference has exceeded the sum of the allowances for error   It must be understood that this *relative chloride balance* is an approximation with respect to increase or decrease in the mean chloride concentration in the body as a whole

B2 and B4 appear to have had definitely a relative chloride loss during the precritical period, B3, B6, and B7, a relative chloride gain, and within the large allowance for error, B1 and B5 had neither clear gain nor loss   During the first 4 days following the crisis a relative gain in chloride was apparent in B1, B3, B4, and B5   In B6 and B7 the diminished chloride intake after the crisis makes it useless to attempt interpretation of that period

It would appear, therefore, that in the pneumonia patients who received a small intake of chloride per day the mean concentration of chloride in the water of the body either remained unchanged or decreased during the precritical period, whereas after the crisis it increased   In those patients who received a larger intake of chloride there was a gain in the mean concentration of chloride in the body before the crisis and either a gain or loss after the crisis

Clinical observations on four lobar pneumonia patients receiving excessive amounts of sodium chloride

Because of the decreased excretion of chloride in the urine sodium chloride has been given by many therapeutically, often by hypodermoclysis   Haden (24) suggested that sodium chloride should be administered orally to all patients with lobar pneumonia to keep the blood chlorides near the normal level   He gave 74 grams of salt in 4 days to one patient in addition to that taken with food   During these 4 days only 7 23 grams were excreted in the urine   The concentration of chloride in the blood was between 62 and 67 mM per liter followed by a subsequent rise to 82 mM , whether related or not to the crisis is not stated   Later Haden (25) reported the clinical course and the blood and urinary chloride analyses in three cases of lobar pneumonia receiving excessive quantities of salt   Except in one case the excessive salt intake was not clearly associated with a rise in the blood chloride

TABLE 3

Serum changes in cases on ordinary diet and in cases receiving extra salt

Case number	Type of organism	Day of disease from crisis	Total base		Serum chloride	Serum protein		Serum specific gravity
			Chemically determined	1 17 × serum corrected conductivity		Serum total nitrogen × 6.25	Serum protein (refractometric)	
			m.Eq per liter	*m.Eq per liter*	*m.Eq per liter*	*grams per liter*	*grams per liter*	*20°C /20°C*
B1	Pneumococcus Type IV	−1	136	140	85	79	74	1 0232
		0	140	144	85	70	76	1 0285
		+2	144	146	85	72	76	
B2	Pneumococcus Type III	−3	149	144	92	70	65	
B3	Pneumococcus Type IV	−3	148	145	87	75	76	
		−2	149	149	91	75	80	
		+1	146	153	89	78	88	
		+3	143	151	94	73	74	
		+6			99	78	81	
		+8	157	156	97	77	78	
B4	Pneumococcus Type II	−2	166	154	99	67	53	
		−1	158	146	88	65	62	
		0	158	142	84	65	60	
		+1		146	86	65	62	
		+3	149	153	93	71	86	
		+5	148	146	95	75	78	
		+7		153	96	76	80	
		+9	148	150	95	76	81	
B5	Pneumococcus Type I	−3		146	91	70	70	
		−1	146	142	87	67	68	
		+1	138	146	86	71	80	
B6*	Pneumococcus Type IV	−5	137	151	83	63	62	1 0235
		−3	143	150	89	65	60	1 0234
		−2	146	150	95	59	56	1 0234
		0	140	149	99	64	59	1 0240
		+2	149	151	95	66	65	1 0239
		+5	149	150	91	72	71	1 0247

* Cases receiving salt therapy

TABLE 3—*Concluded*

Case number	Type of organism	Day of disease from crisis	Total base		Serum chloride	Serum protein		Serum specific gravity
			Chemically determined	1 17 × serum corrected conductivity		Serum total nitrogen × 6 25	Serum protein (refractometric)	
			m.Eq per liter	m Eq per liter	m Eq per liter	grams per liter	grams per liter	20°C /20 C
B7*	Pneumococcus Type III	−6	142	155	90	74	72	1 0255
		−4	143	151	93	80	63	1 0246
		−3	143	152	99	66	60	1 0231
		−1	161	159	108	64	59	1 0235
		+1	155	159	104	64	59	1 0240
		+4	153	152	98	69	62	1 0226
B10*	Pneumococcus Type IV	−5	142	146	93	76	76	1 0281
		−4	155	153	100	71	73	1 0266
		−1	163	169	99	75	84	1 0290
		+1	148	151	101	65	65	1 0230
B11*		−4			91			
		−1			91			
		+1			101			

In a previously reported series of cases (26) no outstanding change was found in the serum chloride concentration or clinical course in patients receiving doses of sodium chloride with the food   A statistical analysis of the serum chloride concentrations in those patients showed no significant deviations from the values observed in patients not receiving extra salt   However, in the present study, where considerably more salt was given, increase in the serum chloride concentration is suggested in three cases   In the patients not receiving salt the concentration of serum chloride decreased until the time of crisis in 6 out of 8 patients (cases reported in 1926 (1) and present cases not receiving salt therapy), whereas, in 3 of the 4 cases receiving large quantities of salt the serum chloride concentration progressively increased until the crisis after which salt administration having been stopped, it fell   This is shown in table 3   With the administration of these larger amounts of salt certain clinical effects have been observed which will be described

In the present group of four patients, each patient received in divided

doses from 15 to 30 grams of sodium chloride per day in the form of a
13 5 per cent sodium chloride solution   The patients objected sur-
prisingly little to these large doses

Abdominal distention

All four of the patients appeared to have less abdominal distention
after salt was given   The abdomen became soft and required less
use of stupes or other measures designed to relieve distention   The
patients took a slightly higher caloric diet than those not receiving
salt, perhaps because of the diminished tympanites   In view of the
increased metabolism in pneumonia, it may be accepted that a higher
caloric diet than is ordinarily given would be desirable provided the
patients could tolerate it   A higher caloric diet without extra salt
was tried in B3 but had to be abandoned on account of the abdominal
distention which developed

Sputum excretion

In 14 precritical observations in pneumonia on patients who were
receiving no chloride therapy the average amount of sputum collected
was 55 grams per day containing 3 7 mM of chloride per day   In
13 precritical observations in patients receiving large quantities of
salt, the excretion of sputum averaged 91 grams per day and contained
11 2 mM of chloride per day   In the patients not given salt the
chloride concentration in the sputum was 67 mM per kilogram,
whereas under larger salt intake it became 124 mM per kilogram, a
higher concentration than was present in the serum   The fact that
the concentration was higher than in serum may simply be the result
of evaporation of water in the respiratory tract   ,

These facts suggest that the retention of chloride observed during the
precritical period when large amounts of salt were given, may in part
be explained by the increase of chloride in the pneumonic exudate

Temperature

The temperature curve in the patients studied appeared to descend
in a general way to a lower level when large doses of salt were given
Whether this was incidental can only be decided on the basis of a
large series   The temperature instead of varying between 101° and

104° became less fluctuating in type and varied between 99° and 101°
No other drugs than sodium chloride and occasionally codeine sulphate
were given to three of the patients, the other, B10, received chloral,
barbital, and sodium bromide on several occasions   In cases B6 and
B7 the temperature did not remain continually within the normal
range until after the first week following what we considered to be
the crisis

Complications

In three of the four patients postcritical pleural collections and
unresolved pneumonic areas were suspected from physical signs and
x-ray studies   The patients presented elevations of temperature and
moderate leucocytosis after the crisis   The signs were never suffi-
ciently definite to necessitate aspiration and the abnormal chest
findings disappeared in the course of about a week   As a result,
however, convalescence was protracted by approximately a week

In studies now in progress there has been substituted for large
doses of NaCl a salt mixture containing per diem KCl, 6 grams,
$NaHCO_3$, 5 9 grams, $MgCl_2$, 1 2 grams   One-half of this mixture when
added to the ordinary food intake of the patient provides a mean
normal salt ration   The caloric value of the diet has also been in-
creased by 500 to 1000 calories with beverages containing 10 per cent
glucose
I wish to express my gratitude to Professor J Harold Austin for
his advice throughout these studies

SUMMARY

The total daily ingesta and egesta have been measured before and
after the crisis in a series of seven patients suffering with lobar pneu-
monia   The body weight, nitrogen and chloride balances were deter-
mined daily   The loss of weight and inadequacy of the diet have been
described and discussed   Partial inanition was an important factor
in inducing the observed loss of weight, although additional factors
appeared to diminish the rate of loss of weight before the crisis and
to increase it after the crisis   The negative nitrogen balance in the
pneumonia patients was higher than in fasting individuals

Patients receiving the usual low caloric diet without extra salt exhibited before the crisis a negative chloride balance, the excretion exceeding the intake This was true in spite of the extremely reduced excretion in the urine After the crisis when more food was being taken, chloride excretion in the urine increased, but, nevertheless, there was usually a positive chloride balance Patients who received a larger intake of chloride during the precritical period either from a more liberal diet or from large doses of salt had precritical retention of chloride.

The chloride balance has been considered also in relation to the change in body weight In patients receiving a small intake of chloride, the mean concentration of chloride in the body appeared to be decreased before the crisis and increased after the crisis, whereas, in patients receiving a larger intake of chloride (5 to 30 grams NaCl daily) the mean concentration of chloride in the body appeared to be increased before the crisis and decreased after the crisis when the chloride administration had been stopped

The present studies of the chloride balance do not support the view that pneumonia is characterized by a retention of chloride during the precritical period, but rather that it is characterized during the pre-critical period by a diminished capacity of the body to conserve chloride on a low intake of chloride and a diminished capacity to excrete chloride on a high intake of chloride After the crisis the chloride balance becomes restored to normal from whichever deviation had earlier developed

The clinical picture observed in four patients who received large doses of sodium chloride precritically has been described

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PROTOCOLS

Case B1 (Number 962)   Italian, male, age 34, butcher   The patient was admitted to the hospital with a consolidation of the left lower lobe   Crisis occurred on the 9th day of the disease   The sputum yielded a Type IV pneumococcus   The leucocyte count was 37,000 with 83 per cent polymorphonuclears on admission   Convalescence was uneventful.

Case B2 (Number 1358)   White, male, age 21, clerk.   The patient was admitted to the hospital on the 6th day of the disease with a consolidation of left lower lobe   He was cyanotic and breathing rapidly   On the 2nd day of hospitalization he became irrational, developed hiccoughs, and died   The blood and sputum both yielded a Type III pneumococcus.   The leucocyte count on admission was 3,000 but became elevated to 16,000 prior to death

Case B3 (Number 1208)   Colored, male, age 40, laborer   Study of this patient was commenced on the 5th day of a left lower lobar pneumonia.   Crisis occurred on the 8th day   During the first 2 days of observation, the patient was given a more liberal diet than usual which had to be discontinued on account of the marked abdominal distention.   The patient perspired profusely on the day of the crisis   The sputum yielded a Type IV pneumococcus   The leucocyte count increased progressively from 20,000 on admission to 32 000 at the time of crisis and then fell.

Case B4 (Number 1046)   Colored, male, age 35, laborer   The patient was first studied on the 6th day of his disease at which time he was very ill and had a consolidation of both lower lobes   Respirations were voluntarily restricted due to pleural pain   Over the left chest pleural and pleuro pericardial frictions were audible   Abdominal distention was marked   The sputum yielded a Type II pneumococcus   Crisis occurred on the 8th day of the disease   The leucocyte count was 34 000 2 days after the crisis   Convalescence was uneventful.

Case B5 (Number 1086)   Colored, male, age 11, school boy   Study was begun on the 7th day of the disease when the patient had consolidation of the entire left lung   The sputum yielded a Type I pneumococcus   Crisis occurred on the 10th day of the disease and was followed by the development of an interlobar empyema.   A thoracotomy was performed and pus drained   The pus contained Type I pneumococcus   The highest leucocyte count was 50 000 and was observed at the time of crisis   Convalescence lasted 6 weeks

B6 (Number 1510)   White, male, age 47, longshoreman   The patient was first studied on the 3rd day of a left lobar pneumonia   The sputum yielded a Type IV pneumococcus   Crisis occurred on the 9th day of the disease   The temperature, however, did not maintain a postcritical normal level but continued to be elevated (99–101°) during the first week after the crisis   Physical signs, x-ray studies, and a leucocytosis of 28,000 at this time suggested the possibility of a pleural collection or unresolved pneumonic areas   These chest findings gradually disappeared without treatment   Convalescence was protracted

This patient had been given large doses of sodium chloride during the precritical period

Case B7 (Number 1491)   Colored male, age 57, laborer   The patient was first studied on the 3rd day of a left lower lobar pneumonia   He was breathing rapidly and appeared toxic   Sputum contained a Type III pneumococcus   A crisis was not definitely observed but was suggested on the 10th day of the disease   Physical signs, x-ray evidence, and a leucocytosis of 15,000 later suggested the possibility of an unresolved pneumonia or pleural collection   These signs, however, disappeared   Temperature reached the normal level one week after the time that was regarded as the crisis   The urine contained on 2 occasions traces of albumin and hyaline and granular casts   Following the intake of large doses of sodium chloride during the epicritical period, the patient showed some puffiness especially of the face and a slight edema of the legs

STUDIES OF SERUM ELECTROLYTES

V Urinary Electrolyte Excretion in Pneumonia[1]

By J Harold Austin and F William Sunderman[2]

(From the John Herr Musser Department of Research Medicine University of Pennsylvania and the Medical Wards[3] of the Pennsylvania Hospital, Philadelphia)

In a previous paper (1) one of us has presented studies of weight and nitrogen and chloride balance in pneumonia   It is probable that the changes studied could have been more readily interpreted had we had full information with respect to the other electrolytes as well as with respect to chlorides   To obtain this was not at the time practical   We have, however, data on the urinary excretion of fixed base, sulphate and phosphate from the same subjects over the same period of days in pooled specimens of urine each representing the combined collection of urine from a subject for those consecutive days during which the chloride excretion had been fairly constant   These data are presented and discussed in this paper   Case numbers are the same as in the previous paper

Total base was measured by Fiske's method (2), inorganic sulphate by Folin's method (3) and phosphate by Briggs' method (4)   The figures for phosphate excretion are expressed as m.Eq which have been calculated by assuming 1 8 m.Eq per mM of PO_4

In order that we might have some standard with which to compare the rates of excretion of these electrolytes in our patients with pneumonia we placed two normal subjects for 2 days on a similar dietary intake and had them undergo severe sweating in a cabinet light bath on each of the 2 days   We have made our comparisons with the second day of study of these normal subjects   Furthermore, in order to compare the patients, who differed greatly in size, all excre-

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[2] Robert M Girvin Fellow in Research Medicine.

[3] Service of Dr George W Norris

tion rates have been reduced to constant surface area by multiplying by the ratio of the surface area of the subject to 2 10   The data are given in Table I

<p style="text-align:center">RESULTS</p>

Cases not receiving extra salt (B1, B3, B4 and B5)   In the total periods of B1 and B5, in the first period of B3 and in the first two periods of B4 the following features are evident when comparing the excretion rates with those of the second day of the control subjects

1   The total water output by all channels was 35 to 96 per cent above the controls

2   The urinary output however, was not increased in two of the four cases although moderately or markedly increased in the other two

3   In contrast to the low chloride excretion the sum of the measured anions excreted was little reduced or actually increased as compared with the controls   When we consider that Holten (5) has demonstrated considerable increase in organic acid excretion in pneumonia the sum of the anions should probably be still further increased relative to the controls   This deserves emphasis as indicating that the very low chloride excretion in the urine in the precritical period of pneumonia is not necessarily paralleled by low *total electrolyte* excretion and it suggests the possibility that the low chloride excretion may be in part an adaptation to make way for other anions the excretion of which is perhaps more important

4   The excretion of phosphate was slightly increased in three of the four cases as compared with the controls

5   The excretion of sulphate was strikingly increased in all four as compared with the controls   The ratio of sulphate to nitrogen excreted was in these periods of the patients and in the controls approximately the same and it seems proper to correlate the increased sulphate excretion with the increased protein metabolism

6   The fixed base excretion was greatly reduced ranging from only 20 to 85 per cent of the mean for the two controls   The low base relative to the anion excretion was presumably compensated for by high ammonia and titratable acid in the urine   Our pneumonia patients in the periods under consideration were therefore excreting an excessive amount of certain acid metabolites under the handicap

of limited excretion of fixed base   At least one factor in the limitation of fixed base available for excretion was undoubtedly the low base intake of the diet

A consideration of these results suggests that the low chloride excretion in the precritical period of pneumonia is dependent in large

TABLE 1

Average excretion per day of measured electrolytes in urine

Case number	Days of period	Total water output	Urine							Surface area
			Water	Chloride	SO₄	PO₄	Measured anions	Fixed base	$\frac{SO_4}{3.61N}$	
		liters	*liters*	*m. Eq*	*m. Eq*	*m. Eq*	*m. Eq*	*m. Eq*		*sq. m.*
B1	−1 +1	5 3	1 26	0	72	43	115	22	0 82	1 68
B3	−3,+1	4 3	1 35	7	78	68	153	86	0 89	} 2 40
	+2 +7	4 1	2 08	88	64	54	206	152	1 01	
B4	−2,+3	5 4	2 01	30	} 85	74	} 194	135	0 88	} 1 91
	+4 +6	4 4	2 48	37		81		142		
	+7, +8	3 8	3 03	176	109	79	364	282	1 25	
B5	−3,+3	6 3	3 66	29	68	34	131	60	0 81	1 02
B6	−5, −4	5 2	2 01	34	154	63	251	62	} 1 02	1 68
	−3,+4	4 8	2 31	338	46	52	436	298		
B7	−6, −5	4 0	1 23	3	61	31	95	52		1 87
	−4, −1	3 8	2 05	83	} 47	34	} 146	80	0 94	
	0,+1	2 4	1 12	48				75		
	+2,+3	2 3	1 19	45				83		
B8	2	3 1	1 09	61	43	49	153	117	0 89	2 05
B9	2	3 3	1 62	58	40	50	148	129	0 87	2 17

* Estimated as 1 8 m Eq per mM

measure on low chloride intake and perhaps in part upon the need for excretion of excessive amounts of sulphate, phosphate, and organic acid under the handicap of little fixed base available for excretion It is possible, furthermore, that this demand for fixed base for excretion is a factor in the low base concentration in the serum in the precritical period of pneumonia. Correlated with this would be a

tendency for lowering of the serum chloride   Furthermore, in the need for transporting the increased amounts of sulphate, organic acids, and phosphate from the tissues to the kidneys we have perhaps a reason, although not a mechanism, for the disproportionate lowering of the serum chloride

In the two patients B3 and B4, studied through more than one period the following features are evident in the final period

7   There was a diminution in the high total output of water associated with increase in the urine and necessarily diminished loss of water by skin and lungs

8   The fixed base excretion was increased

9   The ratio of sulphate to nitrogen increased

10   The chloride excretion increased in correlation we believe, with the increased intake of chloride and fixed base consequent upon the more liberal food intake

Cases receiving extra salt (B6 and B7)   As compared with the patients receiving no extra salt these two subjects exhibited high excretion of fixed base and chloride   In B7 the excretion of chloride was much below the intake and this was associated with marked retention of water and eventually with visible edema   That a tendency to chloride retention characterizes the behavior of patients with pneumonia when given NaCl freely was pointed out in the previous paper (1)

SUMMARY

The low chloride excretion observed in the urine in the precritical period in patients on the usual low diet not receiving salt was associated with low urinary excretion of fixed base but with very high excretion of sulphate and phosphate so that the total anion excretion in the urine was not far from that of normal subjects on a similar diet   The high sulphate excretion was proportional to the high nitrogen excretion and is probably to be correlated with the tissue catabolism

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THE EFFECT OF EXERCISE ON THE SIZE OF NORMAL HEARTS AND OF ENLARGED HEARTS OF DOGS

By HAROLD J STEWART

(From the Hospital of the Rockefeller Institute for Medical Research New York)

Although the changes in the heart produced by exercise have long been made the subject of investigation by clinicians and physiologists, the question has not yet been settled   The earliest view, formulated from clinical experience, was that when the heart was subjected to exertion it dilated   This opinion was derived from estimations of changes in the borders of the heart on percussion (T Schott (1890) and Williams and Arnold (1899))   T Schott (1897) was the first investigator to employ x ray examination in studying this problem The data he collected appeared to support his view that dilatation of the heart occurred   Moritz (1908) in 1908 first demonstrated by means of orthodiagrams that acute cardiac enlargement did not follow over-exertion   Indeed, on the contrary, he found that in certain instances both in normal and pathologic hearts contraction took place   Since then, studies have been made of the effect of many varieties of exertion (swimming, rowing, running in marathon races) on the heart of man, from the point of view not only of discovering whether the heart reacts to acute exertion by dilating or contracting, but also of learning whether hypertrophy is necessarily a consequence of athletic training   More and more, the evidence which has been accumulated seems to indicate that dilatation does not occur as a consequence of acute exertion, but that on the contrary contraction in size takes place   Recently Gordon and Strong (1923) have studied in rabbits the effect of vigorous exercise on the size of the normal and abnormal heart   The hearts designated as abnormal were those in which enlargement resulted from repeated injections of spartein sulphate   According to these authors myocarditis was the resulting pathologic lesion   They found that the effect of exercise was the

same in both instances a decrease in size of the hearts always occurred

We have had an opportunity also to investigate this subject For several years we have been engaged in attempting to find a method which would establish a state in dogs comparable to heart failure in man One of the methods has consisted in the attempt to bring about this state by rendering the mitral valve insufficient We have for this reason in our possession a number of dogs in which defects of the mitral valve have been made by operation and in which enlarged hearts have in consequence developed They show no signs however of heart failure It has been in connection with the study of the circulation of these dogs that we have investigated the response of the hearts to exercise In them we are able to estimate the influence that valvular defect alone (presumably without disease of the muscle) exerts on the reaction of the heart to exertion The form of exercise which we chose was running on a treadmill We took the precaution of studying the effect of the same form of exertion on the hearts of normal dogs These experiments form the subject of this paper

MATERIAL

The subjects of certain experiments, as has been stated, were normal intact dogs The dogs which were the subjects of other experiments were those which had been operated on 2 to 3½ years ago Evidence of the lesions which were then created still existed at the time of the present experiments (table 1) Complete data concerning the operations will be published later (Stewart) A brief description only of the method used in operating on the valves need be given Under ether anesthesia and under aseptic conditions the left auricular appendage was exposed and incised A cardioscope[1] was then inserted through this opening By manipulation of the knife attached to the cardioscope the leaflets of the

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1a

1b

1c

Fig. 1 In this figure are reproduced x-ray photographs showing the effect of exercise on the size of the normal heart. Photograph 1a was taken before and 1b after running. 1c was taken after the dog had rested for 1 hour

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TABLE 1

Effect of exercise on the size of the heart of normal dogs

Dog number	Date	Weight	Cardiac area	Cardiac area per cent of initial	Decrease in cardiac area	Heart rate	Duration of exercise	Distance run	Time with reference to running
		kgm	*sq cm*	*per cent*	*per cent*	*per minute*	*min utes*	*miles*	
235	March 22, 1927	15 2	60 4	100 0		96			Before
			57 3	94 8	5 2	120	45	0 9	Immediately after
			56 1	92 8	7 2	128			One hour after
250	October 26, 1927	10 5	35 8	100 0		120			Before
			34 4	96 1	3 9	110	60	0 8	Immediately after
			34 9	97 5	2 5	130			One hour after
	October 27, 1927	11 0	40 8	100 0		120			Before
			38 9	95 4	4 6	90	60	1 1	Immediately after
			37 2	91 2	8 8	120			One hour after
	October 28, 1927	11 6	36 3	100 0		120			Before
			35 6	98 0	2 0	90	60	1 3	Immediately after
			34 0	93 7	6 3	120			One hour after
	October 29, 1927	10 3	37 9	100 0		85			Before
			35 7	94 2	5 8	85	45	*	Immediately after
			35 6	94 0	6 0	85			One hour after
251	October 27, 1927	12 7	48 8	100 0		110			Before
			46 1	94 5	5 5	90	35	0 4	Immediately after
			46 1	94 5	5 5	115			One hour after

* Speedometer not working

TABLE 2

Enlargement of the heart following induction of artificial mitral insufficiency in dogs

Dog number	Area of heart before operation	Area of heart after operation	Time since operation	Increase in area of heart	Presence of murmur at time of running
	sq cm.	*sq cm*	*years*	*per cent*	
158	46 4	84 4	3₈	81 8	+
161	46 0	72 3	2¼	57 2	+
171	50 3	68 9	2	37 7	⊥
153	56 2	68 2	2¼	21 3	0
90	43 0	42 6	3¼	0 0	0

mitral valve were brought into view The leaflets could then be cut under direct vision. Development of a marked systolic thrill was regarded as evidence that the operation had succeeded The dogs completely recovered within 10 days to 2 weeks The hearts began to increase in size after varying intervals of time A loud systolic murmur persisted in each of the dogs (see exceptions, table 2) The dogs studied for the present purpose were in good health They were trained to laboratory procedures and were not disturbed by them.

METHODS

The dogs were first trained to run on a treadmill Certain dogs ran readily, others could not be induced to run and were not studied After the preliminary training, the effect of running on the size of the heart was investigated by means of x ray photographs of the heart. The x ray photographs were made and measured according to the method described by Stewart (1927) for obtaining photographs of the hearts of dogs under uniform conditions The anticathode was placed at a distance of 2 meters from the photographic film. A suture was inserted in the skin in the mid line of the anterior chest wall at the level of the heart. The anti-cathode was always centered on this point before plates were exposed Three x ray photographs were made during each session the first, before the dog began running, the second, immediately after the dog had stopped running, and the third, after the dog had rested for one hour Since the treadmill was not driven by a motor, but by the dogs themselves, they ran only as long as they did so voluntarily They ran quite steadily for 25 to 60 minutes The second photographs were made as soon as it was evident that the dogs did not wish to run longer The tread was placed at an angle of 19 degrees with the horizontal and a speedometer recorded the number of revolutions from which the distance was calculated. Since the treadmill was in the x ray room only a few seconds were required to transfer the dog from it to the dog board under the x ray tube The x ray photographs were taken within 2 to 3 minutes after the dogs had stopped running The rate of the heart was counted at the apex for one minute immediately after the photographs were taken The observations were usually repeated several times in each dog

The exposures were sufficiently long to photograph the diastolic heart shadow in those photographs in which both systolic and diastolic positions of the heart could be identified, the diastolic area was the one measured

OBSERVATIONS

Effect of exercise on the size of the heart in normal dogs In dog 235 the initial cardiac area was 60 4 sq cm (table 2, fig 1) It decreased to 57 3 sq cm after the dog had been running for 45 minutes After resting 1 hour it decreased still further to 56 1 sq cm There was

TABLE 3

The effect of exercise on the size of enlarged hearts in which systolic murmurs were still present

Dog number	Date	Weight	Cardiac area	Cardiac area per cent of initial	Decrease in cardiac area	Heart rate	Duration of exercise	Distance run	Time with reference to running
		kgm	*sq cm*	*per cent*	*per cent*	*per minute*	*min utes*	*miles*	
158	February 10, 1927	20 6	82 1	100 0		130			Before
			77 2	94 0	6 0	148	60	0 6	Immediately after
			81 9	99 7	0 3	136			One hour after
	March 4, 1927	22 3	87 5	100 0		148			Before
			80 4	91 8	8 2	132	38	0 4	Immediately after
			82 8	94 7	5 3	150			One hour after
	October 13, 1927	19 9	84 4	100 0		90			Before
			79 1	93 7	6 3	120	40	0 5	Immediately after
			80 9	95 8	4 2	114			One hour after
	October 15, 1927	20 1	87 2	100 0		130			Before
			79 9	91 6	8 4	110	57	0 6	Immediately after
			83 7	96 0	4 0	130			One hour after
161	January 28, 1927	16 0	72 3	100 0		160			Before
			65 9	91 1	8 9	142	38	0 3	Immediately after
			71 0	98 2	1 8	144			One hour after
	February 9, 1927	16 8	73 6	100 0		122			Before
			68 3	94 1	5 9	142	25		Immediately after
			68 5	94 4	5 6	134	31	0 2	Immediately after
			74 1	100 6	0 6				One hour after
	October 13, 1927	14 5	72 0	100 0		104			Before
			68 3	94 8	5 2	104	30	0 3	Immediately after
			71 2	98 8	1 2	120			One hour after
171	March 14, 1927	20 5	68 9	100 0		140			Before
			66 9	97 1	2 9	130	60	0 8	Immediately after
			62 8	91 2	8 8	148			One hour after
	March 15, 1927	21 3	64 5	100 0		140			Before
			60 5	93 8	6 2	142	44	0 8	Immediately after
			59 0	91 5	8 5	140			One hour after
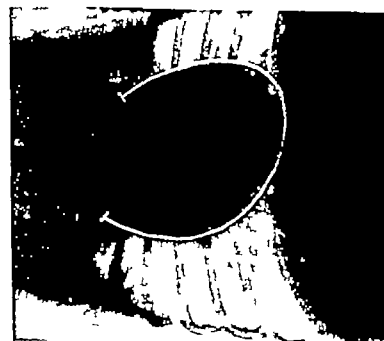
FIG 2 In this figure are reproduced x ray photographs which show the effect of exercise on the size of the enlarged heart Photograph 2a was taken before and 2b after running 2c was made after the dog rested for 1 hour

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accordingly a fall to 94 8 per cent of the initial size followed by a further decrease to 92 8 per cent of the initial size one hour after exercise

There are 4 observations on dog 250 and one on dog 251 (Table 2) The results in all instances are similar to the one just reported The size of the hearts decreased 3 9 to 5 8 per cent immediately after running, but decreased still further 6 0 to 8 8 per cent 1 hour later In not a single instance did the size of the heart become greater than it had been in the beginning

Effect of exercise on the size of enlarged hearts in which systolic murmurs are present Mitral insufficiency was created in dog 158 on December 2, 1924, 3¿ years ago During this time the area of the heart increased 81 8 per cent from 46 4 sq cm to 84 4 sq cm (table 2) This was the area on October 13, 1927 (table 3, fig 2) After running 40 minutes on this day it decreased to 79 1 sq cm , 93 7 per cent of the value before exercise One hour later it was 80 9 sq cm , 95 8 per cent of what it was before the start There was then on this occasion a decrease of 6 3 per cent in the size of the heart after exercise The dog ran on three other occasions On these also the cardiac area decreased, the maximum decrease varying between 6 0 and 8 4 per cent

Mitral insufficiency was created in dog 161 on December 11, 1924, $2\frac{1}{6}$ years ago During this time the area of the heart increased 57 2 per cent from 46 0 sq cm to 72 3 sq cm (table 2) This was the cardiac area on January 28, 1927 (table 3) After running 38 minutes on this day it was 65 9 sq cm , that is to say, it had diminished 8 9 per cent One hour later it was 71 0 sq cm , approximating the size it was before the start The dog ran on two other occasions On these also a decrease in size of the heart occurred The maximum decrease amounted to 5 9 per cent and 5 2 per cent respectively

Mitral insufficiency was created in dog 171 on April 22, 1925, 2 years ago During this time the heart had enlarged 37 7 per cent from 50 3 sq cm to 68 9 sq cm (table 2) He ran on the treadmill on two occasions The decreases immediately after running were 2 9 and 6 2 per cent respectively and were 8 8 and 8 5 per cent respectively one hour later (table 3)

There are observations therefore on 3 dogs in which the heart was

enlarged and in which there was still evidence that defects of the mitral valve (murmurs) were still present. In every instance the size of the heart decreased following the exercise of running on a treadmill

Effect of exercise on the size of the heart in dogs subjected to operation but in which there was no longer evidence that valvular defect was present Mitral insufficiency was created in dog 153 on November 20, 1924,

TABLE 4

Effect of exercise on the size of the hearts of dogs subjected to operation but in which systolic murmurs were no longer present

Dog number	Date	Weight	Cardiac area	Cardiac area per cent of initial	Decrease in cardiac area	Heart rate	Duration of exercise	Distance run	Time with reference to running
		kgm.	*sq cm.*	*per cent*	*per cent*	*per minute*	*min-utes*	*miles*	
153	January 18 1927	23 0	69 6	100 0				0 6	Before
			66 7	95 8	4 2		42		Immediately after
			62 9	90 4	9 6				1 hour after
	January 22, 1927	23 0	67 8	100 0		182		0 6	Before
			60 9	89 0	11 0	145	55		Immediately after
			67 9	100 0		170			1 hour after
	January 25, 1927	23 0	68 2	100 0		165		0 4	Before
			62 5	91 5	8 5	170	50		Immediately after
			67 5	99 0	1 0	155			1 hour after
90	April 15, 1927	11 5	42 6	100 0		130		0 8	Before
			40 2	94 3	5 7	120	38		Immediately after
			42 6	100 0		112			1 hour after

2⅔ years ago   During this time the size of the heart increased 21 3 per cent, from 56 2 sq cm to 68 2 sq cm (table 2)   A systolic murmur was heard for some months after operation but later it disappeared   It was not heard at the time of these experiments   The dog ran on three occasions   On each occasion the heart was smaller after running than it had been in the beginning (table 4)   It decreased 8.5 per cent on the first, 11 per cent on the second and 4 2 per cent on the third occasion   One hour later it had nearly regained its

initial size on the first, it had returned to its initial size on the second, and decreased still further (9 6 per cent) on the third occasion

Mitral insufficiency was created in dog 90 on February 28, 1924 A soft systolic murmur was heard for some months after operation but later it disappeared Three and one sixth years after operation the area of the heart was approximately the same as before operation (0 8 per cent decrease) (table 2) The area of the heart was 42 6 sq cm on April 15, 1927 (table 4) After running on the treadmill for 38 minutes it was 40 2 sq cm, that is to say, a decrease of 5 7 per cent had occurred The initial size was regained one hour later

In one dog (3 occasions) then the subject of an artificially enlarged heart and in a second in which there was no enlargement, the hearts decreased in size after running on a treadmill The maximum decrease was 11 per cent A systolic murmur was not heard in either case

Effect of exercise on the heart rate The heart rate decreased after running in 2 normal dogs (dog 250, 3 occasions and dog 251, once) (table 2), unchanged once (dog 250) and increased once (dog 235) In the case of the dogs in which the hearts were enlarged, the rate sometimes decreased (dog 158 twice, dog 161 once, dog 171 once, dog 90 once, and dog 153 once), sometimes increased (dog 158 twice, and dog 161 once) and was sometimes unchanged (dog 161 once, dog 171 once, and dog 153 once) It is clear that the same effect was not always observed even in the same dog

DISCUSSION

We have presented data showing that the hearts of normal dogs decrease in size following exercise Decrease in size also occurred in hearts that were enlarged, whether the valve defect (murmur) was or was not still present The effect in dogs in which the hearts were enlarged was the same as in the normal hearts, the dogs were all in good health and exhibited no evidence of heart failure Although the mitral valves had been subjected to damage and the hearts had enlarged in consequence of this injury, there had occurred no disease of the heart muscle so far as is known Although the decrease in size was small, 3 9 to 11 per cent, it is nevertheless significant and it occurred consistently Enlargement never was observed In the hour after exercise the hearts sometimes decreased still further in size

(if the decrease in the first instance was small), sometimes remained unchanged and at other times regained their initial size   We have no explanation to offer for this difference. The changes which we observed in dogs were not as great as those observed in rabbits by Gordon and Strong (1923)   The difference in the results obtained is probably due to the fact that the rabbits ran until completely exhausted, while our dogs ran only as long as they did so voluntarily The distance which the dogs ran may appear to be small   The average distance was 0 6 mile, but the inclination of the tread (19°) necessitated a vertical ascent of 1000 ft in this distance

We cannot be certain of the factors responsible for the occurrence of the decrease in size of the hearts of these dogs after exercise   It was observed also to accompany regular tachycardia (Stewart and Crawford, 1927)   Decreased filling of the heart in the shortened diastole may be one of the factors   In the case of these experiments however tachycardia was not a factor, since the ventricular rate exhibited no consistent change, remaining unchanged or becoming either slower or faster than the initial rate. Increase in size of the vascular bed may of course have taken place due to the opening of channels which were hitherto closed, for exercise may be believed to be the occasion for the opening of a greater number of capillaries in the muscles and skin than when the animals are in a resting state   The blood would then be drained away from the heart.

Both Meek and Eyster (1922) and Stewart (Stewart, a) have shown that the size of the heart decreases when the volume of circulating blood is diminished   But decrease in volume of blood was in all probability not a factor in these cases since Hastings (1921) has shown an increase following exercise in the hemoglobin content (expressed as oxygen capacity) of the blood and Broun (1922) has demonstrated an actual increase in the total blood volume

In another connection also there is similarity in behavior of large and small hearts   The same dogs which served in these experiments were utilized also in studying the effect of digitalis   In both groups decrease in cardiac size, decrease in cardiac output and increase in ventricular excursion followed the administration of this drug (Cohn and Stewart, 1928a and 1928b)

It is not certain how far an application can be made of the data of

these experiments to cases of heart disease in man   The analogy is not close enough to warrant the conclusion that acute exercise is not harmful to patients suffering from valvular disease when they exhibit no signs of heart failure   It is infrequent that one finds in patients the conditions which were present in these experiments, that is to say, valvular insufficiency and cardiac enlargement without concomitant disease of the myocardium

SUMMARY

The effect of running on a treadmill on the size of the hearts of dogs has been studied   It was found that the size of both normal and enlarged hearts always decreased

CONCLUSIONS

Dilatation of the heart does not occur in normal dogs following voluntary exercise   On the contrary, the size of the heart decreases When dogs in which the heart is enlarged in consequence of artificially created valvular defects, but in which there is presumably no myocardial disease, and in which there are no signs of heart failure, are subjected to exercise, the size of the heart likewise decreases

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METABOLISM OF CHLORIDE AND TOTAL FIXED BASE IN PNEUMONIA AND THE RELATION TO SALT AND WATER RETENTION

By T. S. WILDER and T. G. H. DRAKE

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Although much has been written regarding an unusual behavior of chloride and certain other ions in pneumonia, the literature fails to supply a clear cut conception of the sequence of events in the metabolism of acid and base in this disease. Largely because investigations have tended to consider only a few factors at a time it is difficult to evaluate their relationship to other elements in the situation. Several findings have, however, been thoroughly established, and it is the object of this paper to attempt an explanation of them in terms of certain accompanying circumstances. An underlying variable, the effect of which we have particularly studied, is the level of salt and water intake.

Peabody (1) (2), in his papers of 1912 and 1913, summarized the previous literature and reported the observations that most patients with pneumonia have low serum and urinary chlorides, and a high urine ammonia. He reported also a decreased elimination of Na, Ca, and Cl, but a normal or increased excretion of K and Mg, inferring therefrom a retention of the former ions but not of the latter. By analysis of tissues, Peabody went on to show that there is nowhere an accumulation of the "retained" ions, which led him to believe that these must be spread diffusely through the body.

McLean (3) in 1915 confirmed Peabody's findings and noted also that the low serum chloride found in pneumonia returned to normal before the low urinary chloride showed a similar change.

In 1916 Maver and Schwartz (4) observed the presence of pitting edema in some cases of pneumonia and not in others, but felt that their

EXPERIMENTAL

Patient W H was studied for the 3 days preceding and the 6 days succeeding his crisis During the first 3 days he gained nearly 2 pounds in weight, which same amount he lost immediately after the crisis Determinations of the chloride and total base of the serum showed those values to be normal two days before the crisis, and at that time there were also 104 and 241 cc N/10 respectively of those ions in the 24 hour urine Following the crisis there was a sudden large increase of acid and base radicals, chiefly chloride and sodium, in the urine, the maximum values being 1641 cc N/10 of chloride, and 1791 cc N/10 of base on the second day after the crisis Coincidently, the volume of the urine nearly doubled This patient therefore, although acting in the expected manner with regard to his weight changes, did not show either a low blood chloride and base, or a low urinary excretion of these ions at the height of his fever

The second patient, A S , was studied for 5 days preceding his crisis and 9 days succeeding it *He lost weight*, to the extent of $3\frac{1}{2}$ pounds, during the first 8 days, (i e 5 days *before*, and 3 days *after* the crisis), and although on a nearly zero salt intake this patient excreted 1084 cc N/10 base, and 442 cc N/10 chloride in urine and stools, during the $6\frac{1}{2}$ days he was on the metabolism frame In the blood serum three determinations of base and four of chloride showed steadily decreasing values prior to the crisis and a definitely low chloride as late as 36 hours after the crisis The urinary values for base and chloride decreased rapidly before the crisis and remained exceedingly small for 4 days thereafter, that is to say for over 2 days after the weight had begun to rise The patient was put on a full milk diet 48 hours after the crisis, but it was not until 3 days later that base and chloride appeared in the urine in moderately large amounts, amounts nevertheless which did not approach the values achieved by the first patient during his phase of maximum post-critical excretion In table 1 are given figures which show the extent of loss of base and chloride in the first two periods, (i e while on a very low salt intake), and the markedly positive balance of these ions after a salt containing diet was resumed (It is perhaps noteworthy that base and chloride appear, throughout, to behave very much in

the same way, and it need hardly be mentioned that by far the largest part of the base referred to is sodium)

From the preceding it is evident that the behavior of the 2nd patient was exactly opposite to that of the first To be sure his serum and urinary chloride and base dropped to low levels, but contrary to the

TABLE 1

Average base and chloride balances per 24 hours in patient A S

	Period I (36 hours) low salt diet	Period II (5 days) low salt diet	Period III (6 days) high salt diet
Fixed base cc. $N/10$			
Intake.	0	0	1 433
Output in urine	130	27	285 5
Output in stools	300	61	206 5
Total output	430	88	492 0
Balance	−430	−88	+941
Chloride, cc. $N/10$			
Intake	0	0	374
Output in urine	152	13	144
Output in stools	48	15 5	17
Total output.	200	28 5	161
Balance	−200	−28 5	+213
Water, cc.			
Intake.	875	854	1 284
Output (urine and stools)	509	490	858
Difference	+366	+364	+426
Weight, grams			
Average daily gain or loss	+40	−157	+83

conventional idea this patient lost much weight and showed a large negative balance of both base and chloride previous to the crisis Chart 1 illustrates graphically the contrast between the findings from these two patients The effort on the part of patient A S to restore salt to the body is shown by the early rise of the weight curve through

Patient W H was studied for the 3 days preceding and the 6 days succeeding his crisis   During the first 3 days he gained nearly 2 pounds in weight, which same amount he lost immediately after the crisis   Determinations of the chloride and total base of the serum showed those values to be normal two days before the crisis, and at that time there were also 104 and 241 cc N/10 respectively of those ions in the 24 hour urine   Following the crisis there was a sudden large increase of acid and base radicals, chiefly chloride and sodium, in the urine, the maximum values being 1641 cc N/10 of chloride, and 1791 cc N/10 of base on the second day after the crisis   Coincidently, the volume of the urine nearly doubled   This patient therefore, although acting in the expected manner with regard to his weight changes, did not show either a low blood chloride and base, or a low urinary excretion of these ions at the height of his fever

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Fixed base cc. N/10			
Intake	0	0	1,433
Output in urine	130	27	285 5
Output in stools.	300	61	206 5
Total output.	430	88	492 0
Balance	−430	−88	+941
Chloride, cc. N/10			
Intake	0	0	374
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Total output.	200	28 5	161
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Water, cc.			
Intake.	875	854	1,284
Output (urine and stools)	509	490	858
Difference	+366	+364	+426
Weight, grams			
Average daily gain or loss	+40	−157	+83

conventional idea this patient lost much weight and showed a large negative balance of both base and chloride previous to the crisis Chart 1 illustrates graphically the contrast between the findings from these two patients. The effort on the part of patient A S to restore salt to the body is shown by the early rise of the weight curve through

immediate retention of salt and water, and the late appearance of salt
in the urine when placed on a moderately high salt diet

We soon realized that the key to the difference shown by these two
infants lay in the fact that the former received a moderately large
amount of NaCl during the course of his illness and retained water,
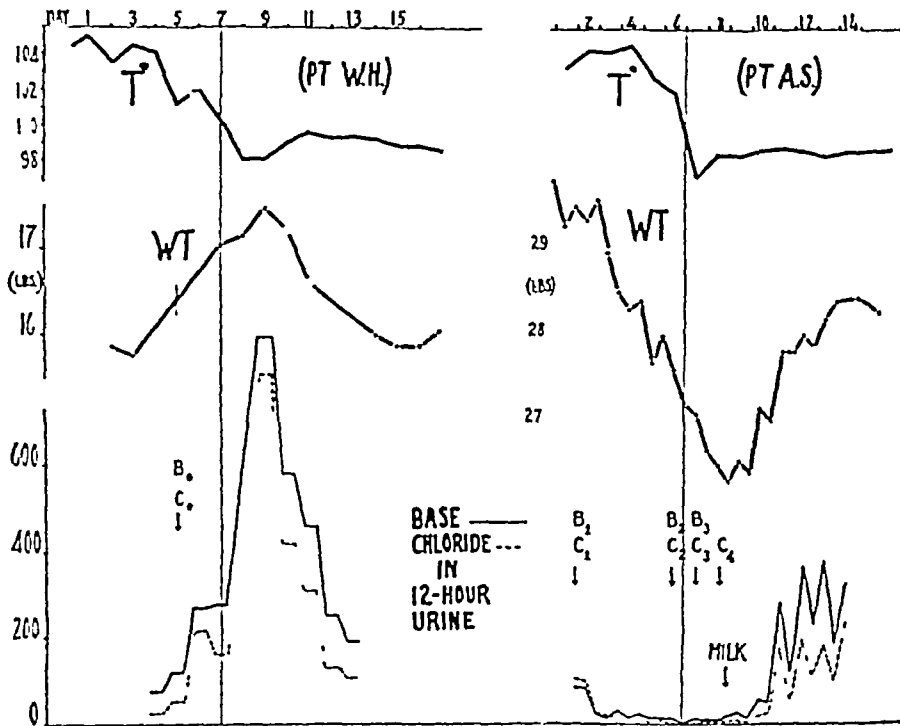
CHART 1 ILLUSTRATING DIFFERENCES IN WEIGHT CURVES AND IN URINARY
    EXCRETION OF CHLORIDE AND BASE, BETWEEN PATIENT W H ON A MODER-
    ATELY HIGH SALT INTAKE AND PATIENT A S ON A VERY LOW SALT INTAKE

Crisis occurred at points marked by vertical lines B_0 = 153, B_1 = 157,
B_2 = 149, B_3 = 137 cc N/10 Base in the blood serum, C_0 = 96, C_1 = 96, C_2
= 90, C_3 = 85, and C_4 = 93 cc N/10 Chloride in the blood serum

whereas the latter was given almost no salt and as a result lost weight
despite an average daily intake of over 1200 cc of fluid in the form
of orange juice and glucose solution

This surmise was abundantly confirmed by a survey of the cases of
primary pneumonia in the records of the Infants' Hospital   Taking

first a group of infants whose diet had been restricted to orange juice and glucose solution, we found an average salt intake of only 0 7 gram a day or the equivalent in a 20 pound infant of about 6 cc N/10 NaCl per pound body weight   Like patient A S described above, these patients lost much weight previous to the crisis   See chart 2

In contrast with the above group were other patients which had made gains of 30 to 40 ounces in as short a time as 36 hours   These
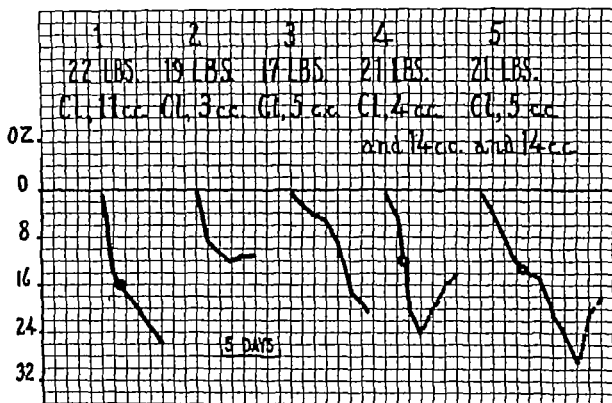
CHART 2 ILLUSTRATING EXTENT OF WEIGHT LOSS OBSERVED IN PATIENTS ON LOW INTAKES OF SALT, EXPRESSED AS CHLORIDE

Dotted line represents weight curve following increase of salt intake   Circle represents crisis

were all found to have received large amounts of salt in their diets, usually in the form of a mixture of normal saline solution (0 9 per cent), and orange juice   Some of these infants took 6 grams of salt a day, or from 50 to 75 cc. N/10 NaCl per pound body weight, in other words about ten times the amount obtained by the group on a low salt intake   See chart 3

In addition to the primary differences in the weight curves of infants on low and infants on high salt intakes, charts 2 and 3 illustrate

in certain patients the effect of changing from one diet to another
In chart 2 may be noted two infants, (no 4 and no 5), who lost weight
while on salt intakes of 4 and 5 cc N/10 respectively per pound body
weight, but who gained rapidly as soon as the intake was increased to
14 cc   The reverse effect is seen in chart 3   Patients no 2, no 4,
and no 5 gained in weight when getting 59, 32, and 30 cc N/10 NaCl
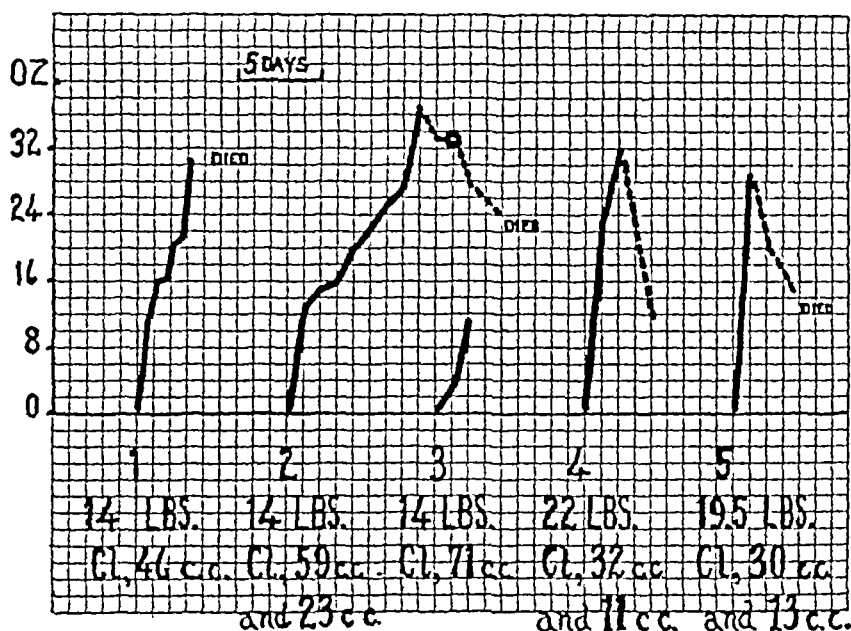
CHART 3 ILLUSTRATING EXTENT OF GAINS IN WEIGHT OBSERVED IN PATIENTS
ON HIGH INTAKES OF SALT, EXPRESSED AS CHLORIDE

Dotted line represents weight curve following decrease in salt intake   Circle
represents crisis

per pound body weight, and then lost when the salt was reduced to
23, 11, and 3 cc respectively
From these findings it seems clear that the patients represented by
chart 2 could not retain water until they were also provided with a
certain level of salt intake   Like A S they lost weight, and like him
no doubt they also had a negative base and chloride balance at the
time   A negative balance indeed is strongly suggested in these
patients by the fact that their weight loss continued beyond the crisis

until after their salt intakes were increased, indicating that in them there had been no appreciable storage of salt that could be released at the crisis as a sort of endogenous supply   In other words it appears that the hypothesis of chloride retention in pneumonia is incorrect when applied to the group of patients on low chloride diets that show low blood and urine values of base and chloride and are losing weight   Extensive retention of Na and Cl, however *does* occur on a high salt diet but in this event there will be found a normal or high level of base and chloride in the serum, with considerable amounts of these ions entering the urine, chemical findings again, which are contrary to current theories

A third metabolic study, (included as case no 1 in chart 3), may be mentioned briefly here as a patient that received, under our observation, a relatively large amount of salt in his diet, (i e 46 cc N/10 NaCl per pound body weight), and furnished us with some significant post-mortem data   Details of the acid base metabolism and of tissue analyses will form the subject of a succeeding paper, but it is of interest to note here the principal findings, namely, normal serum chloride and base, high urinary chloride and base, rapidly increasing weight with pitting edema, a positive balance of Na and Cl ions, great retention of water and salt in the form of edema fluid, and a marked "retention sec," in the body tissues in general   As the patient was a Mongolian idiot with a congenital lesion of the heart, death was perhaps due to that condition   The grave potential dangers, nevertheless, of high salt therapy are suggested by the intense edema that developed in this infant, a finding that was also recorded in the autopsy reports on patients no 3 and no 5 of chart 3   Although not conclusive evidence against salt therapy it is at least suggestive that among 19 cases of primary pneumonia in infants on whom we could get adequate data, the three deaths that occurred were confined to the small group of five cases that received large amounts of salt in their diet, and as noted above, developed extensive edema of their tissues

There is little evidence to suggest that edema in these patients might have been due to disease of heart or kidneys, either functional or organic. Indeed, marked efficiency of these organs in the patient mentioned above is indicated by the fact that the concentrating power of the kidneys was high throughout the illness   Parallel with the

increasing elimination of salt, both the concentration and the actual amounts of chloride and fixed base in the urine mounted steeply without showing signs of having approached a maximum prior to death

Why the tissues of the body are so markedly at the mercy of the salt intake in pneumonia, despite normally functioning kidneys is not clear   A hint of a specific effect referable to the pneumococcus is offered by the fact that intense general edema sometimes develops in the presence of a pneumococcus abscess, only to vanish when the abscess is drained   On the other hand it is possible that other febrile diseases do manifest in lesser degree the same sort of phenomena, described above, that in pneumonia are rendered especially striking because of the contrasts this disease furnishes in its abrupt onset and its termination by crisis

DISCUSSION

The data given in this paper deal principally with the relation in pneumonia of salt intake to salt and water retention, and appear to show that the latter depends largely on the former

Leiter (14) has already pointed out that any study of salt metabolism must take into consideration the salt intake for several days previous to the study   This generalization we believe applies to primary pneumonias, and the reason so many cases described in the literature have a low blood chloride when first seen may well be that these patients correspond with our infants on low salt intakes by virtue of having refused or neglected to take salt-containing foods during the prodromal stage of the illness, previous to admission to the hospital   Findings in this type of case have come to be regarded as characteristic of the disease only because the majority of pneumonia patients desire water and fruit juices (or are offered nothing else), and so become established on a nearly salt-free diet

The opposite situation of high salt intake reveals in pneumonia an obligatory retention of salt and water which is most striking   In extreme cases the edema becomes so great as to be possibly dangerous It is also conceivable that the incidence of effusions into serous cavities is greater in patients on high than in patients on low salt diets   Consequently it has become our practice to observe caution with salt in pneumonia, giving enough however to keep the patient from losing

weight, namely, about 15 cc. N/10 NaCl per pound body weight, per 24 hours This quantity is supplied by a solution made up of 1 part saline (0 9 per cent), 2 parts orange juice, and three parts 10 per cent glucose, provided the patient takes 2 ounces of the mixture per pound body weight in 24 hours, thus far cases of pneumonia so treated have maintained nearly stationary weights, and have not developed edema

CONCLUSIONS

1 In infants with primary pneumonia we find a negative base and chloride balance if the intake of these ions is low, and a markedly positive balance if the intake is high

A. In the former situation one observes low values for total base and chloride in the serum, rapidly diminishing values for these ions in the urine, rapid loss of weight, and later a gain in weight only after the salt intake has been adequately increased

B In the latter situation the serum values are at least normal, the base and chloride of the urine are normal or high, weight rises rapidly before the crisis, (often with visible edema), and after the crisis there is usually a sharp loss in weight accompanied by an out-pouring of chloride and base in the urine

2 An extensive and perhaps a dangerous degree of edema is often seen to develop in patients who receive large quantities of salt during the early stages of their pneumonia These patients, despite normal heart and kidneys, appear to be forced to retain in their tissues amounts of salt which a normal individual would excrete without difficulty

3 In an attempt to maintain an even weight in patients with pneumonia, we have administered 15 cc N/10 salt solution per pound body weight per twenty-four hours, with satisfactory results

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AN INVESTIGATION OF VARIOUS FACTORS WHICH AFFECT THE SEDIMENTATION RATE OF THE RED BLOOD CELLS[1]

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Since the work of Fahraeus, in 1921 (1), the phenomenon of the settling of the red corpuscles in blood to which an anticoagulant has been added has been studied intensively by biologists as well as by clinicians   The former have been interested chiefly in determining the mechanism whereby the settling rate of the cells is increased or diminished in certain physiological and pathological conditions, and in studying the various chemical and physical factors which may vary the rate, while the clinical investigators have attempted to evaluate the diagnostic and prognostic significance of observed changes in the rate of sedimentation   Preliminary to a renewed effort along clinical lines, we have carried out a series of experiments designed to show the effect which certain factors may have upon the settling rate.

The sedimentation tubes used in these experiments contained 1 0 cc of blood, were 4 0 mm in diameter, were sealed flat at one end, and were graduated from 0 to 80 mm   They were coated on the inside with a very thin layer of paraffin   All blood samples were collected without stasis and were manipulated entirely under oil [2]

THE EFFECT OF VARIOUS ANTICOAGULANTS UPON THE RATE OF SEDIMENTATION

The precipitation of colloids of the suspensoid type by adsorption of an electrolyte and the stabilizing of an otherwise labile system by

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[1] A portion of the experimental work was completed in 1926 at the Henry Ford Hospital, Detroit.

[2] These tubes were 20 mm shorter than those finally decided upon and described for clinical use in the J Clin Invest., 1928, v, 531

a similar process are familiar phenomena   Fahraeus (1) noted that, if defibrinated horse blood is diluted with a 0 9 per cent solution of sodium chloride, the specific gravity and viscosity of which are less than that of the serum, the sedimentation rate of the erythrocytes is actually diminished   Furthermore, he showed that if as little as 0 025 gram of solid sodium chloride is added to 5 0 cc of defibrinated horse blood, the sinking of the cells is decreased from 75 to 3 mm in 15 minutes, and that there occurs an increase in the negative charge on the corpuscles, proportional to the amount of sodium chloride added   In these instances, the suspension has been stabilized by addition of the electrolyte   Popper and Kreindler (2) have likewise shown the stabilizing effect of the addition of electrolytes to the blood   On the other hand, Linzenmeier (3) and Fahraeus (1) have both shown that the sedimentation rate increases if small amounts of lanthanum nitrate are added, and that when larger amounts of this salt are introduced the corpuscles of human blood become positively charged   Provided the magnitude of this charge is sufficiently large the system can be made stable   The precipitating effect of some salts and the stabilizing effect of others is probably referable to the valency of the cation, as pointed out by Oliver and Barnard (4)

Because of the changes that added electrolytes may bring about in the stability of the blood, it seemed advisable to determine the effect of the different anticoagulants in common use, and to adopt for clinical work a substance which has no effect upon the suspension stability other than keeping the blood from clotting ⌠ Sodium fluoride (50 mgm per 5 0 cc of blood), potassium oxalate (14 mgm per 5 0 cc of blood), and potassium citrate (10 mgm per 5 0 cc of blood) were taken as representative of the inorganic anticoagulants, and were compared with each other and with heparin (1 mgm per 5 0 cc of blood), the organic anticoagulant obtained from liver substance   In every instance the concentration of anticoagulant was sufficient to prevent clotting during the period of the experiment⌡

Chart 1 presents graphically the varied rates of settling found when separate specimens of the same blood were treated with the different anticoagulants in the amounts indicated above   With the settling most rapid in the heparinated sample, it is apparent that the rate of sedimentation in the others varies inversely with the concen-

tration of salt necessary to prevent coagulation   Similar differences were noted in several other experiments of the same nature. No attempt was made to determine whether the slowing varies mathematically with the concentration of the salts

In the Linzenmeier (3) method employed by a great many investigators for the clinical determination of the sedimentation rate, one part of 5 per cent sodium citrate is used to four parts of blood   Such
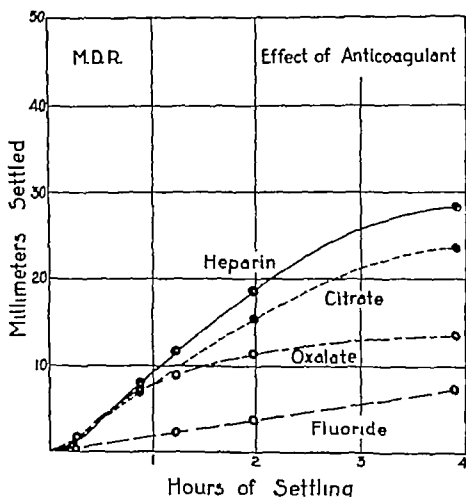
CHART 1 THE EFFECT OF DIFFERENT ANTICOAGULANTS UPON THE SEDIMENTATION
RATE OF BLOOD CELLS

a mixture was compared with blood treated with dry potassium citrate (10 mgm per 5 cc of blood) and with heparin (1 mgm. per 5 cc of blood)   The results are shown in chart 2   The diluted blood (III the curve) settled more slowly even than the blood treated with dry citrate. In the diluted specimen, the concentration of citrate was equal to 50 mgm. in 5 cc. of blood, a fact which undoubtedly explains the slower settling   The dilution itself would increase

the rate, so that the real effect of the increased salt concentration is actually much greater than is apparent

It now became essential to learn whether heparin has some active influence toward hastening the settling, or whether the results obtained when this substance is used represent the sedimentation rate of untreated blood   It seemed reasonable to assume that if a certain concentration of any anticoagulant affects the stability of the blood,
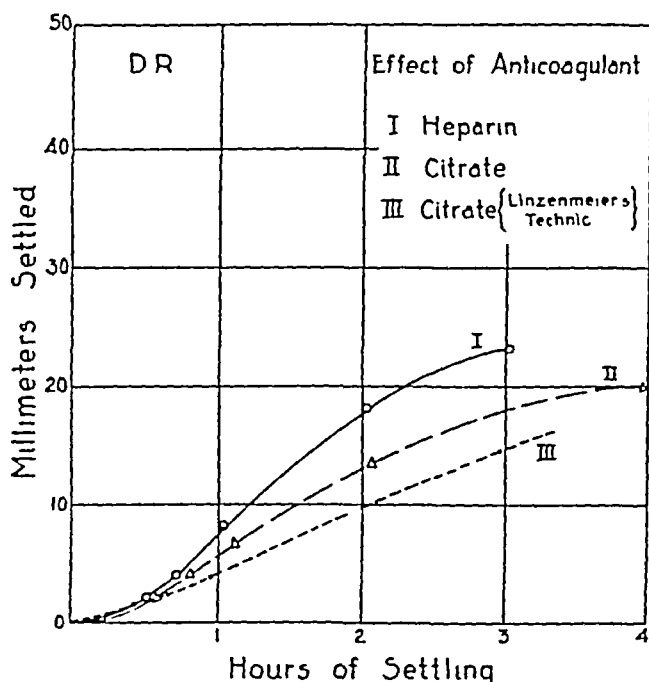
CHART 2 THE EFFECT UPON THE SEDIMENTATION RATE OF SOLID CITRATE AND CITRATE SOLUTION AS COMPARED WITH THE EFFECT OF HEPARIN

a greater concentration should produce a more marked effect in the same direction   Doubling the concentration of the oxalate, the citrate, or the fluoride always produces an appreciably greater slowing of the sedimentation rate, whereas, even when the concentration of heparin is tripled or quadrupled there is no effect upon the rate of settling (chart 3)

It is obvious that the concentration in the plasma of the anticoagulant will be less in those bloods with high plasma concen-

percentages than in those with higher hematocrits For this reason, the stabilizing effect of the electrolyte anticoagulants varies from one blood to another, and the results are not strictly comparable Heparin should then be the preferred anticoagulant for this type of

Rate Of Sedimentation
Of Red Blood Cells

Effect Of Changing Concentrations
Of The Anticoagulants
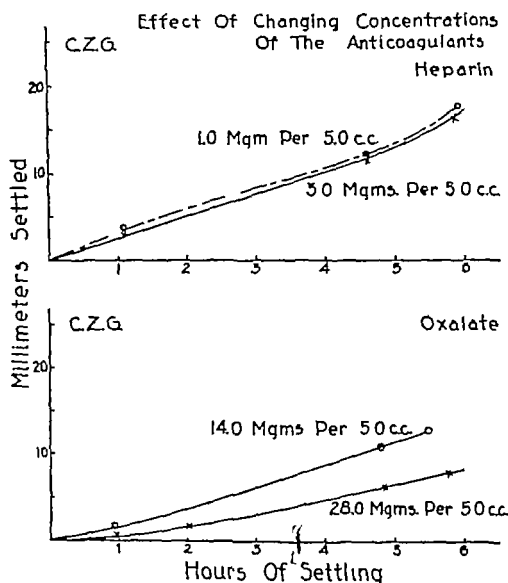
CHART 3 THE EFFECT UPON THE SEDIMENTATION RATE OF INCREASING THE
CONCENTRATION OF DIFFERENT ANTICOAGULANTS (OXALATE AND HEPARIN)

work, for this substance even in considerable concentration does not affect the sedimentation rate. Heparin was, therefore, used as the anticoagulant in all the experiments reported below

Absolute proof that heparin does not affect the sedimentation rate was not easy, but finally we were fortunate enough to secure a

sample of blood from a hemophiliac, whose clotting time was over five hours   Chart 4 shows the sedimentation curves in two specimens of this blood, the one with no anticoagulant and the other with heparin (1 mgm per 5 cc of blood)   Since there was no demonstrable differ- ence in the settling rates of the two samples, we were forced to the conclusion that heparinated blood settles more rapidly than blood treated with fluoride, citrate, or oxalate not because heparin accelerates

P W
Hemophiliac

Effect of Anticoagulant
o Heparin
x No Anticoagulant

Millimeters Settled

Hours of Settling
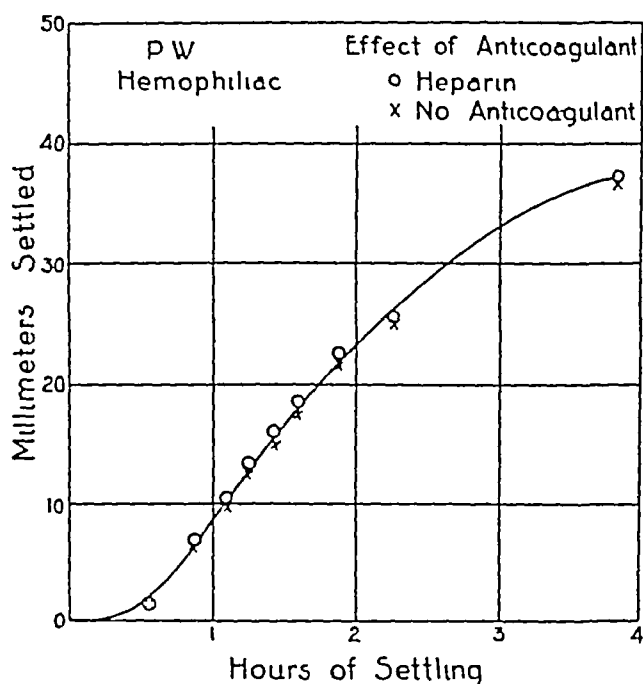
CHART 4 NO EFFECT UPON THE SEDIMENTATION RATE OF HEMOPHILIAC BLOOD
WHEN HEPARIN IS ADDED

settling, but solely because it has no appreciable effect, whereas the electrolyte anticoagulants produce a definite slowing

CHANGES IN THE STABILITY OF THE BLOOD AFTER STANDING AT ROOM TEMPERATURES

Pohle (5) states that if blood is allowed to stand and is then re- shaken, the sedimentation velocity is rather markedly accelerated as compared with freshly drawn blood Westergren (6), however,

found that preserving blood for several hours at room temperature does not materially affect its settling velocity, but that after twenty-four hours standing the rate is slowed   Fahraeus (1), working with defibrinated horse blood reports that a specimen may be allowed to stand at room temperature for as long as seven hours without changing

TABLE 1
Heparin—our technic

Freshly drawn	Millimeters settled in one hour after standing							
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours
42		41		37		36		33
44	44	43	44					
40		40	39	40	40			
39		39	39	39	38			
35		35	33	35				
48		46	46	46	46			
19		20		21		21		22
8		7		8		8		13
6		5		7		6		6
5		5		5		6		6

TABLE 2
Sodium citrate solution—Linzenmeier's technic

Freshly drawn	Millimeters settled in one hour after standing								
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	12 hours	24 hours
15			12		12		13	12	
11			11		10		10	10	
12			10		9		10	8	
4			4		3		4	4	
35		35		34		35			21
53		53		53		54			44
46		45		44		44			38
48		49		49		49			48

the amount of rouleau formation or the sedimentation velocity, but that when such blood is kept at higher temperatures (up to 58°C) rouleau formation is diminished, with a minimum at 42°C   When he allowed a sample of oxalated blood to stand for 5 hours at 28°C and another similar sample at 18°C for the same period, the former

settled more slowly   Wiltshire (7) has shown that oxalated human plasma loses more and more of its aggregating power on standing at room temperature   Obviously, it is of importance to learn definitely how much time may elapse between the collection of blood and the completion of the sedimentation test without any sacrifice of accuracy

The following experiments were performed to measure changes in stability on standing with our method of preventing coagulation with heparin, as well as with Linzenmeier's method using one volume of 5 per cent sodium citrate to four volumes of blood   The bloods stood at temperatures of 22° to 25°C and settling took place at the same temperatures

⌊Blood tends to become more stable on standing, but within the period of a working day the changes are usually insignificant, while after twenty-four hours at room temperature a considerable slowing of the sedimentation rate is usually observed   The effects of heparin and sodium citrate solution are practically the same⌋

Such results lead us to conclude that, for clinical purposes, blood sedimentation determinations may be postponed for some hours (6 to 12) after the blood is drawn without any considerable loss of accuracy, but that standing for a longer period may lead to serious errors by slowing the settling rate

We are at a loss to explain why our results and Westergren's do not agree with those of Pohle, for we have found the reaction completely reversible within the time limits noted   Our blood samples were manipulated entirely under oil, but since Westergren does not state that he took a similar precaution, it seems doubtful whether that factor can explain the varied results

THE EFFECT ON THE RATE OF SEDIMENTATION OF CENTRIFUGING AND REMIXING THE CELLS AND PLASMA

It was necessary in some of our experimental work to centrifuge the blood and later remix cells and plasma for a second determination of the sedimentation rate   To determine whether such manipulations have any effect upon the settling velocity, several specimens were treated in this way and the sedimentation rates compared with those of the original samples

Experiment 1   E L M , normal young woman   A specimen was

set up at once for determination of the sedimentation rate and the remainder immediately centrifuged for 20 minutes at 2500 r p m This latter specimen was then remixed and the rate measured   The settling curves for the two specimens are identical   (See chart 5)

Experiment 2   Dog A.   Distemper   The blood was treated as in experiment 1 and the settling curves are identical (chart 5)   It is

Effect of Centrifuging at 2500 R.R.M. for 20 Minutes.

o- Untreated Blood
Δ-Same after Centrifuging and Remixing.

Dog A.

E.L.M.

Hours of Settling

(y-axis: Millimeters Settled; x-axis: 1, 2, 3, 4)
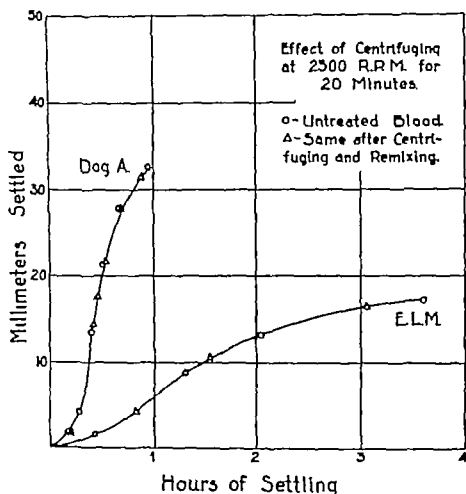
CHART 5 No EFFECT UPON THE SEDIMENTATION RATE AFTER CENTRIFUGING BLOOD FOR 20 MINUTES AT 2500 R.P M. AND REMIXING

important to note that rapidly-settling bloods are as reversible after centrifuging as are normal bloods

Experiment 3   M D R , normal young woman   The blood was treated as in experiment 1, except that after centrifuging the second sample and before remixing, the blood was allowed to stand for two hours at room temperature   The untreated specimen settled 14 mm in one hour, while the other settled 15 mm —an insignificant difference.

We have done other experiments of this character as well as a

limits   In a typical experiment reducing the carbon dioxide content from 69 to 7 per cent increased the sedimentation rate from 20 to 22 mm in one hour, while increasing the oxygen content from 6 to 16 per cent increased the settling from 9 to 11 mm in one hour   Although these changes are very small they are consistent with several other
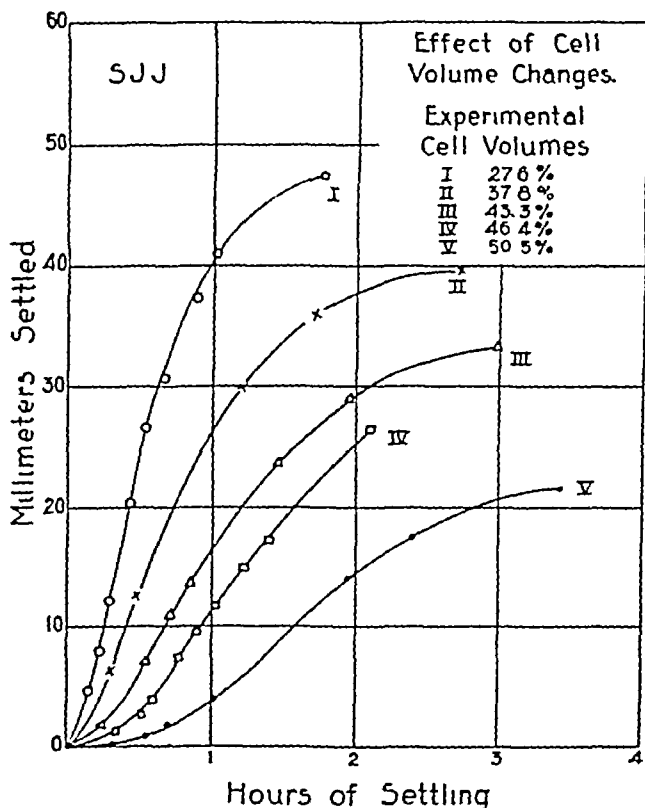
CHART 7   THE EFFECT UPON THE SEDIMENTATION RATE OF A SLOWLY SETTLING BLOOD PRODUCED BY CHANGING THE CELL VOLUME

experiments recorded by this author   It would seem that simple aeration should produce only insignificant variations

Our experimental procedure was as follows Four cubic centimeters of blood were drawn from an arm vein under oil, using heparin as the anticoagulant, and the sedimentation rate was determined as

and agitated by rotating until the blood had become bright scarlet in color, after which its sedimentation rate was determined by the usual procedure   The time of reading was varied so that packing was not a factor

The results of this study are in accord with what might be expected from the experiments of Popper and Kreindler (2), and of Ito (15)
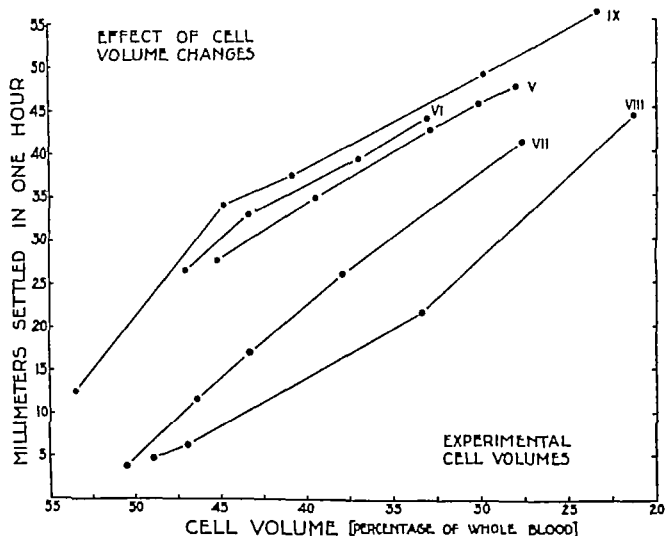
CHART 8 THE MILLIMETERS SETTLED IN ONE HOUR PLOTTED AGAINST THE CELL VOLUME

Five experiments in which the cell volume was artificially regulated

We conclude that the sedimentation rate is not appreciably affected by aeration or by loss of carbon dioxide, and that for practical clinical purposes collection under oil is not necessary, although this precaution would seem indicated where unusual accuracy is desirable. This conclusion would seem applicable to both slowly and rapidly settling bloods

THE EFFECT OF TEMPERATURE UPON THE SEDIMENTATION RATE

DeCourcy (8), using the Linzenmeier technic, determined the sedimentation rates of various blood samples at 6°, 18°, and 37°C and concluded that while there was only a slightly increased rapidity of settling between 6° and 18°C, there was a considerable increase between 18° and 37°C Westergren (6) shows graphically the variations in sedimentation rates at different temperatures, 14° to 25°C, of bloods settling with various speeds, and suggests the use of similar curves for correctional purposes He also found that the effect of an increase in temperature is smaller with slowly settling

TABLE 3

The effect of aeration of the blood upon the sedimentation rate

Blood number	Time settled	Millimeters settled	
		Sample as drawn	Aerated sample
1	1 hour	10	10
2	1 hour	11	14
3	1 hour	12	12
4	1 hour	6	4
5	1 hour	8	9
6	1 hour	3	3
7	1 hour	9	8
8	20 minutes	32	32
9	20 minutes	28	31
10	10 minutes	33	33

bloods and that the effect of a definite (e g, a 5°C) variation in temperature is greater as the temperature increases Gordon and Cohn (16), using Linzenmeier's method, studied the sedimentation rates at 10°, 23°, and 37°C and found startling differences in all the rates between these temperatures They also found that the effect of external temperatures within these limits is transitory, i e that upon shaking and retesting the blood at a second temperature no influence of the original temperature will be apparent These authors conclude that since the average room temperature varies between 22° and 24°C, a standard of 23°C should be maintained during the determination of the settling rate

We have determined the sedimentation by our method[3] on specimens of the same samples of blood at different temperatures varying from 20° to 38°C , and our findings in the main are similar to those noted above   Carefully regulated water baths were used for all the determinations, and the samples were allowed to remain in the baths for 30 minutes before the tests were started in order to obtain tempera ture equilibrium   Chart 9 shows graphically the curves obtained in
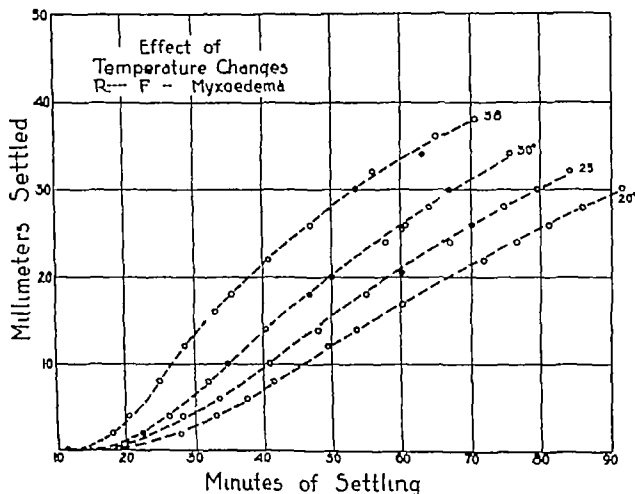
CHART 9 THE EFFECT OF TEMPERATURE CHANGES UPON THE SEDIMENTATION RATE

a typical experiment where the blood was maintained at four different temperatures ranging from 20° to 38°C   The findings in 13 other experiments are presented in table 4

It is obvious that the settling rate increases to an appreciable extent with rising temperature within the range with which we are dealing, and that the increase in rate is less from 20° to 25° than from 25° to

[3] Tubes giving a column of blood 100 mm high have been used for these tem perature experiments   (See J Clin Invest., 1928, v, 531)

30°C Furthermore, the relative increase of rate per degree of temperature from 30° to 38° is greater than from 20° to 25° in the slowly settling specimens

In more rapidly settling bloods, however, packing becomes such an important factor in the one hour readings at 38° that the true temperature effect is masked and the curve falls off In specimen 13, infectious arthritis, packing has almost entirely masked the temperature change

TABLE 4

The effect of temperature changes on the sedimentation rate

Subject	Sex	Diagnosis	Millimeters settled in one hour at			
			20°	25°	30°	38°
I C W M	M	Normal			2	3
II W M	F	Normal	2	2 5	4	6
III M R	M	2nd and 3rd degree burns			6	11
IV D G	F	Normal	6	7	10	14
V Dog B	F	Normal			9	14
VI B J	M	Normal	9	11	15	20
VII R R	M	Normal	12		19	23
VIII R F	F	Myxedema	17	20	26	34
IX B M	M	Tuberculosis (mild)	23	26	30	33
X L G	M	Rheumatic fever	28	32	39	40
XI H H	M	Arsphenamine dermatitis	37	39	46	48
XII R F	M	Tuberculosis	40		46	48
XIII A R	M	Infectious arthritis	59	59	59	60

We can not suggest the use of curves similar to those presented in chart 9 for correctional purposes, since the type of curve for rapidly settling bloods is dependent upon the relative importance of the packing factor, which would produce a greater straightening of the temperature rate curve for bloods with higher hematocrits (greater cell volumes)

For clinical work, we suggest that all tests be carried out at a room temperature of 20° to 25°C , since this range is not difficult to maintain in most laboratories, and since we feel that the variations in rate at such temperatures are not great enough to affect the value of

the results   Any apparatus, which might be used to control the
temperature more closely (constant temperature baths or rooms)
would not be practical for clinical work, and so can not be advocated
However, if tests must be made under unusual temperature condi
tions, such as extreme summer heat, an attempt should be made to
control the temperature between 20° and 25°C , or due allowance must
be made in interpreting results   It is obvious that it is only in middle
range (doubtful cases) that the effect of temperature changes might
interfere with the interpretation of results

THE EFFECT OF INGESTION OF FOOD UPON THE STABILITY OF THE BLOOD

Sedimentation rates and plasma protein fractions (Wu's (17)
method) were determined upon blood samples from two subjects
immediately before and shortly after the ingestion of food   In
another experiment (no 3), the sedimentation rate was determined
at 11 00 a m and at 4 00 p m , the subject not having eaten since
8 00 a.m   In the last experiment (no 4) blood samples were taken
for the determination of settling rates four hours after breakfast and
again four hours after lunch on the same day

The changes noted are quite insignificant and confirm DeCourcy's
(7) observations   It seems doubtful whether there is any reason to
prescribe that tests for clinical purposes should be run at any particu
lar time of the day

THE EFFECT OF SHORT, VIOLENT EXERCISE UPON THE SEDIMENTATION RATE

The effect of muscular exercise in concentrating the blood (in
creased plasma protein percentage and decreased plasma volume
percentage) has been thoroughly studied by Scott, Hermann, and
Snell (18), Peters, Bulger, Eisenmann, and Lee (19), and Rowe (20)

In view of this concentration, it would be reasonable to expect that
there would be some alteration of the settling rate of the blood cells,
but we can find no record of an experimental investigation of this
point.   Our experiments were made upon healthy, young individuals,
blood samples being collected just before and immediately after the
exercise period.   Plasma proteins were determined by the method of
Wu (17), the plasma carbon dioxide by the Van Slyke and Stadie

TABLE 5

Effect of ingestion of food upon stability of the blood

Experiment 1　　E S , normal young woman

Specimen	Plasma				Cell volume	Settling after ½ hour
	Fibrin	Globulin	Albumin	Total protein		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	
No 1—11 30 a m , 4 hours after breakfast	0 34	2 66	4 64	7 64	44 9	13
No 2—1 30 p m , 1 hour after lunch	0 31	2 62	4 54	7 47	42 9	16

Experiment 2　　C W M , normal young man

Specimen	Plasma				Cell volume	Settling after ½ hour
	Fibrin	Globulin	Albumin	Total protein		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	
No 1—11 30 a m , 3½ hours after breakfast	0 32	2 42	4 95	7 69	46 9	3
No 2—1 30 p m , 1 hour after lunch	0 30	2 60	4 93	7 83	45 0	3

Experiment 3　　M D R , normal young woman

Specimen	Cell volume	Settling after ½ hour
	per cent	
No 1—11 00 a.m , 3 hours after breakfast	42 7	14
No 2—4 00 p m , no lunch, 8 hours after breakfast	43 2	17

Experiment 4　　J L M , normal young woman

Specimen	Cell volume	Millimeters settled in 1 hour
	per cent	
No 1—11 30 a m , 4 hours after breakfast	43 6	6
No 2—4 30 p m , 4 hours after lunch	43 6	6

procedure (21), and the sedimentation rate and cell volume on heparinated blood according to our own technic.

Short, violent exercise of this nature tends to concentrate the blood,

increasing the plasma protein percentage, which should, according to our concept, make for more rapid settling of the cells, and at the same time increasing the cell volume percentage, which should retard sedimentation   The observed decrease in the carbon dioxide content of the plasma should have no appreciable effect on the sedimentation

TABLE 6

Effect of exercise upon the sedimentation rate

Experiment 1   R. L J , a healthy young man ran up and down four flights of stairs four times, taking three minutes for the task, and being quite exhausted at the end

	Plasma				CO₂ content	Heparin cell volume	Settled in 1 hour
	Fibrin	Globulin	Albumin	Total protein			
	per cent	*per cent*	*per cent*	*per cent*	*volumes per cent*		
Before exercise	0 25	2 10	4 80	7 15	49 0	45 5	6
After exercise	0 27	2 32	4 95	7 54	38 0	48 0	8

Experiment 2   M D R., a healthy young woman, ran up and down three flights of stairs six times in four minutes

	Plasma				Heparin cell volume	Settled in 1 hour
	Fibrin	Globulin	Albumin	Total protein		
	per cent	*per cent*	*per cent*	*per cent*		
Before exercise	0 31	2 12	4 40	6 85	45 6	7
After exercise	0 35	2 22	4 68	7 25	48 1	6

Experiment 3   P M H , a healthy young woman ran up and down three flights of stairs three times in two minutes

	Heparin cell volume	Settled in 1 hour
Before exercise	40 4	11
After exercise.	44 2	11

velocity, according to the studies of Ito (15)   Assuming that other factors, such as increased lactic acid, have relatively negligible effects, it would seem that, in the experiments noted, the increase in plasma proteins, particularly the fibrin fraction, produces an accelerating effect, which is approximately (in experiment 3 exactly) equalized

by the retarding effect of the increase in cell volume percentage The subject warrants more study, but in the light of these experiments, we feel that the sedimentation velocity of the blood cells could not be altered materially by any usual state of activity of the individual

CONCLUSIONS

1 Heparin is the ideal anticoagulant for blood sedimentation studies, since it has no effect on the settling rate other than keeping the blood from clotting The inorganic anticoagulants depress the rate of settling in proportion to their concentration Dilution with the citrate solution described in Linzenmeier's technic slows the rate to a greater extent than does the dry salt

2 Aggregation of the red blood cells under conditions obtaining in sedimentation tests is a completely reversible phenomenon Blood may usually stand at room temperature for six to twelve hours without the sedimentation rate being affected materially, whereas longer standing generally leads to a slowing of the rate All tests should be completed on the day when the blood is drawn

3 Centrifuging blood for 20 minutes at 2500 r p m does not affect its settling velocity after remixing

4 Blood dilution (with its own plasma) leads to more rapid settling of the cells In samples where the aggregating and packing factors are not largely operative this change is represented by a straight-line curve

5 Aeration of venous blood has no significant effect upon the rate of settling, therefore the collection of blood samples under oil is not necessary

6 Increase of temperature (within certain limits) makes for more rapid sedimentation Within the range of ordinary room temperatures, the changes are hardly significant

7 The changes in rate of settling due to the ingestion of food are slight and may be disregarded for ordinary work

8 Short, violent exercise has an insignificant and variable effect on the sedimentation rate The accelerating effect of the fibrinogen increase is compensated by the retarding effect of the increase in cell volume

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OBSERVATIONS ON THE EFFECT OF VARIOUS FACTORS ON THE DURATION OF THE ELECTRICAL SYSTOLE OF THE HEART AS INDICATED BY THE LENGTH OF THE Q-T INTERVAL OF THE ELECTROCARDIOGRAM

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INTRODUCTION

The duration of systole, both absolute and relative to diastole, has long been a subject of interest to physiologists, but it has been almost wholly ignored by clinicians   In fact many physicians are not even aware that the duration of systole varies so much as it does with heart rate and that the first and second heart sounds are on some occasions twice as far apart in time as on other occasions   A considerable physiological literature relative to the duration of systole exists, but very little is found in the clinical literature   Fridericia (1) (2) and Mili (32) are the only writers who have discussed the subject in any detail from the clinical point of view, that is, as to the effects of various pathological conditions on the duration of systole   The one finding of common knowledge physiologically and known to the few clinicians who have been interested in the subject is that systole shortens appreciably with increasing pulse rates, but less rapidly than does diastole

It has seemed to us worth while to gather more data on the effect of various clinical conditions on the duration of electrical systole to supplement the scanty information that we have at present   Hence we have made measurements of electrical systole in a large group of patients with various disease conditions as well as in a group of normal controls

The duration of electrical systole we have measured from the electrocardiogram (the interval between the beginning of the Q wave

and the end of the T wave) as will be recounted below　Although this interval has not coincided with the measurements made on many mechanical curves, it is possible and in fact probable that it represents more accurately the true duration of systole, provided good measurable curves are obtained, than does any mechanical record　At least this interval is a reliable index of the duration of systole and can be used in a study of the relative effects of various conditions with a high degree of confidence in its reliability for such comparisons

<div align="center">LITERATURE</div>

Fridericia (1) in 1920 published a paper on the duration of systole under normal conditions as measured by the electrocardiogram, which method he considered reasonably accurate　He referred to early measurements of systole both by mechanical records and by electrocardiogram　He concluded from his own study and from observations in the literature that "with rising pulse rates systole and diastole shorten but the latter more "　In measuring the Q-T interval of the electrocardiogram, he found the Q or R onset usually sharp with an accuracy of 0 001 second　The end of T was accurate in suitable curves to 0 01 second　If the P wave fell on the T wave, measurement was, he thought, impossible　Variations in the leads were such that he always used lead II　Average errors were not over 0 005 second

He carefully selected and studied 50 normal people mostly between 20 and 40 years old, 28 male and 22 female　He found the following variations of the duration of systole with pulse rate

<div align="center">Table of Fridericia's normal findings (expressed in fractions of a second)</div>

Pulse rate	Number of cases	Average Q T interval	Limits of Q-T interval
		seconds	
50–60	4	0 405	0 420–0 398
60–70	10	0 370	0 402–0 344
70–80	11	0 353	0 373–0 325
80–90	7	0 339	0 352–0 327
90–100	8	0 335	0 360–0 321
100–110	5	0 318	0 340–0 308
110–120	1	0 302	
120–130	3	0 290	0 312–0 273
Over 130	1	0 280	

From these data Fridericia constructed the following formula for the determination of the normal average duration of the Q-T interval of the electrocardiogram (systole)　$S = 8\,22\,\sqrt[3]{p}$, in which S = Q-T interval, p = pulse period in hundredths of a second, and 8 22 = constant

Fridencia also investigated the effects of various drugs and poisons on the duration of ventricular systole. Among other findings he reported that when the heart rate was increased by adrenalin, systole was shortened much less than when the tachycardia resulted from muscular work.

A later paper the same year (1920) by Fridencia (2) reported his findings in the study of the duration of systole in the electrocardiogram by the use of his formula under various abnormal conditions   He concluded that a deviation greater than 0 045 second from the expected duration of systole as determined by his formula was the outer limit of normal   Two hundred and eleven curves of 124 patients were studied with measurements made from lead II   He found two chief diffi cultes in measurement, first in arrhythmia, and the 30 cases showing arrhythmia he considered separately, and second in the presence of abnormalities of the T wave (absence, inversion, diphasic nature)   He found that inversion of the T wave shortened systole.

Of 94 patients with normal cardiac mechanism, Fridencia found only 65 whose electrocardiograms were satisfactory for measurement   Of these 65 only 7 showed an abnormal duration of systole   The diagnoses in these seven cases were, mitral stenosis 2, aortic insufficiency 1, aortic stenosis 1, aortic and mitral in sufficiency 1, myocarditis and scoliosis 1, and chronic nephritis with hypertension and uremia 1   Fridencia concluded that heart muscle weakness and increased work for the heart caused prolonged systole in these cases   However, hyperten sion, mitral stenosis, and aortic valve disease generally in this series gave normal Q-T intervals

Of 30 patients with arrhythmia 5 cases of auricular fibrillation were unmeasur able and the T wave was negative in 9 more.   In 15 cases with positive T waves all showed normal Q T intervals except five, too long in four and too short in one The cases with overlong Q T intervals showed many extrasystoles in one, complete heart block in two and partial heart block in one   In one case of paroxysmal tachycardia the Q T interval was found too short but normal in two others with the same mechanism

Fridencia's papers are of considerable interest and confirm conclusively the observation that electrical systole shortens with increasing heart rates but less than does diastole   The application of his formula to clinical cases and the con clusions drawn at times from rather scanty material demanded further investiga tion of the subject.

Miki (32) added in 1922 clinical data to Fridencia's observations, but further study has seemed to us necessary to determine whether or not there is any practical application clinically for the determination of the duration of systole from the electrocardiogram

Miki studied the electrocardiograms of 178 people and applied the three formu las of Fridencia (1), Bazett (30) and Lombard and Cope (26) to them all.   There was a great variation in these cases between the various formulas, that of Lombard and Cope being least in agreement   Among the 178 cases there were one of aortic

stenosis, 9 of aortic regurgitation, 10 of mitral regurgitation, 10 of mitral stenosis, 3 of pulmonic stenosis, 12 of combined valve lesions, 20 of left ventricular hypertrophy, 1 of right ventricular hypertrophy, 7 of cardiac dilatation, 7 of "myocarditis," 10 of exophthalmic goiter, 1 of myxedema, 21 of nervousness, 7 with normal hearts and 5 with bradycardia without block   In all this group there were only 13 cases which gave a value of the duration of the Q-T interval more than the limit allowed by Fridericia's formula and these 13 cases were not particularly significant except that most of them had rapid rates (exophthalmic goiter, exercise, nervousness)   Of 21 cases of auriculo-ventricular block, 16 gave results longer than the calculated figure from Fridericia's formula while only 9 were greater than Bazett's formula allowed   Miki concluded that in dissociation the duration of systole may become abnormally long and that with damage to heart muscle there tends to be a shortening of systole rather than a lengthening, as Fridericia had believed

Other workers, mainly physiologists, have as already stated, studied the duration of systole and references to their publications are listed at the end of this paper   It may merely be noted here that the first who made observations on the shortening of systole with increasing pulse rates from pulse tracings were Garrod (3, 4, 5, 6) in 1870 to 1875, Thurston (7) in 1876, Landois (8) in 1876, Waller (9) in 1887, Edgren (10) in 1889, Stockmann (11) in 1889, Kraus (12) in 1891, Lüdentz (13) in 1892, Einthoven and de Lint (14) in 1900, and Zuntz and Schumburg (15) in 1901   Since then there have been many papers published concerning the duration of systole as measured from pulse tracings or electrocardiograms, but there has been little of the clinical application of the measurement of systole

METHOD OF PRESENT STUDY

During the past three years the electrocardiograms of 213 individuals, 50 normal and 163 abnormal, have been studied by us to determine the influence of various factors on the duration of electrical ventricular systole   Only good records with clearly defined Q-R-S and T waves were measured   Lead II was generally selected for measurement but if the ventricular deflections were higher and more clearly defined in leads I or III, either of these other leads was used   A comparison of the time intervals in the various leads was made in several cases and as a rule the lead with the highest T wave gave the best indication for measurement of the Q-T interval   Generally such a lead gave also somewhat longer Q-T intervals but usually within the limit of error allowed in measurement

The duration of electrical systole was considered to be the time interval between the onset of the Q-R-S wave and the end of the T wave   The use of this measurement for the duration of systole has

already been discussed by a number of workers and we believe that even if the Q-T interval may not exactly indicate the duration of systole at least it is a satisfactory index of it and accurate for comparative studies   As we have already remarked we believe that this Q-T interval may actually record the duration of systole better than does any other measurement

In each case the duration of the Q-T interval was measured for three or more successive heart cycles and then averaged, the heart rate was determined by measurements also of the T Q intervals giving us thus the total cycle lengths   The Lucas comparator was used for all the measurements   Time intervals were figured in ten thousandths of a second but finally recorded in thousandths of a second   They are accurate we believe down to one-hundredth of a second   The electrocardiograms were made almost invariably in the sitting position, rarely in the recumbent position

The results have been charted as seen in the figures   Because of the difficulty and dissatisfaction with the use of any formula, simple curves have been constructed to show the normal limits of the duration of electrical systole according to the chief factor influencing it, namely pulse rate   The findings in groups of cases representing a variety of different conditions have been plotted against these normal control curves   We believe that this method serves better than any formula to show when systole is abnormally long or short, and to demonstrate the fact in a simple graphic way   We have plotted in addition to our own 50 normal control cases, 190 normal cases from the literature (those giving satisfactory Q-T measurements), giving us thus a total of 240 normals for comparison with our abnormal cases

RESULTS

I Normal cases

1 Results at rest In figure 1 is seen the chart of our 50 normal control cases   There is evident a definite relationship between duration of electrical systole in thousandths of a second marked off by horizontal coördinates, and heart rate in beats per minute marked off by vertical coördinates   Thus at rates between 60 and 70 the normal range of systole in our cases was from 0 400 to 0 314 second, at rates between 90 and 100 it was from 0 356 to 0 280, and at rates between
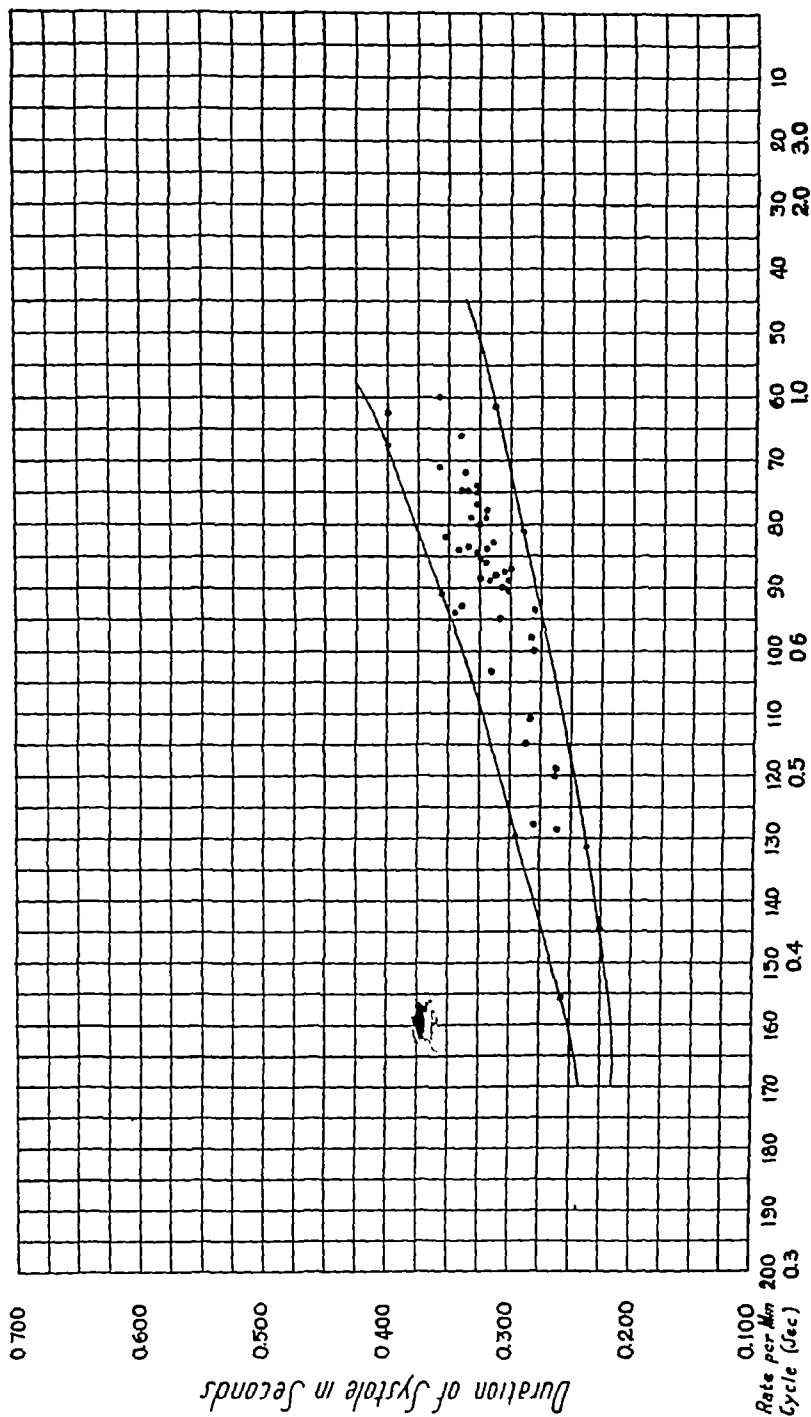
Fig 1 Chart Giving the Duration of the Q-T Interval of the Electro cardiogram According to Pulse Rate in 50 Normal Subjects Including 20 Men, 15 Women, and 15 Children

Each dot represents one case   Heart rate and cycle length are shown in horizontal coördinates and duration of the Q-T interval in thousandths of a second is shown in vertical coördinates   All the succeeding charts are similarly constructed   The limits of measurement of these 50 normal cases of our own series are connected by curves giving a comet shaped track

120 and 150 from 0 295 to 0 225 second   The limits of these measurements are joined, resulting in a comet shaped track, narrow at fast rates   These 50 normal cases included 20 men, 15 women and 15 children (both boys and girls)   For this figure and those that follow the duration of the heart cycle is plotted horizontally as well as heart rate, being 2 0 seconds for a rate of 30, 1 0 second for a rate of 60, 0 6 second for a rate of 100, 0 5 second for a rate of 120, and 0 4 second for a rate of 150

In figure 2 is seen the chart of the 190 normal cases from the literature (Fridericia, (1), Mılı (32), Kraus and Nicolai (16), Seham (42), Bazett (30), Katz and Feil (36), and Fenn (35)).   They include men, women and children   On this chart in dotted lines are superimposed the curves of figure 1   It will be seen that the limits in figure 2 are somewhat greater through most of the chart than those in figure 1   The coördinates represent as in figure 1 the duration of systole and heart rate   On all the charts to follow, these same coördinates are indicated and the combined limit curves of the 240 normal cases of figures 1 and 2 are superimposed so that at a glance one can tell the situation in a given condition   In the chart of the normal cases from the literature shown in figure 2, the duration of systole at heart rates of 40 to 60 varies from 0 480 to 0 308 second, at rates of 80 to 100 from 0 390 to 0 270 second, at rates of 120 to 130 from 0 312 to 0 230 second, and at rates of 150 to 160 from 0 240 to 0 220 second   Two cases at extremely fast heart rates (180 and 198) fall outside the projected limits, one above and one below

2 Normal subjects in different positions   Five normal subjects were electrocardiographed in three positions—lying, sitting and standing   In every instance with one exception, the pulse rate rose as the position was changed from recumbent to sitting, and in each instance without exception, the pulse rate rose further on changing from the sitting to the standing position   As will be seen in figure 3 the Q-T interval measurements show that the duration of electrical systole varies exactly as does the pulse rate   The normal limit curves from figures 1 and 2 are charted on figure 3

It would appear that the effect of gravity in cutting down the blood inflow volume in the right heart, as reported by Lombard and Cope (41), is not necessary in these five subjects discussed here to explain
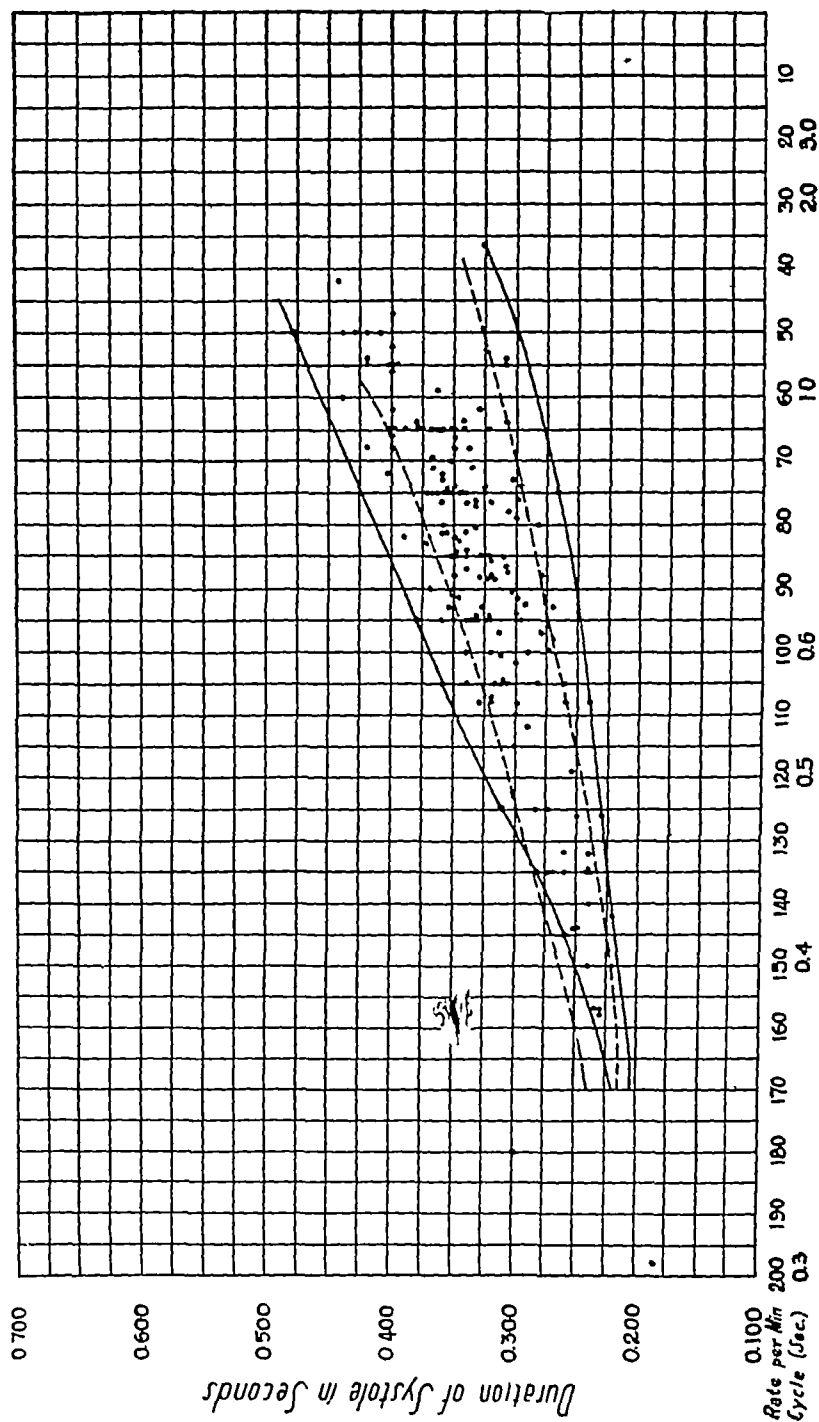
FIG. 2 CHART GIVING THE DURATION OF THE Q-T INTERVAL OF 190 NORMAL INDIVIDUALS PUBLISHED IN THE LITERATURE The outer limits of measurement of these cases are connected by solid lines and the limit lines of our own series of 50 normal cases are superimposed as interrupted lines   Two cases at very rapid rates are seen to fall outside these limit lines when projected

In all figures to follow the combined outer limit lines of these 238 normal cases are charted and are used as an indication of normal or abnormal length of Q-T interval at varying heart rates
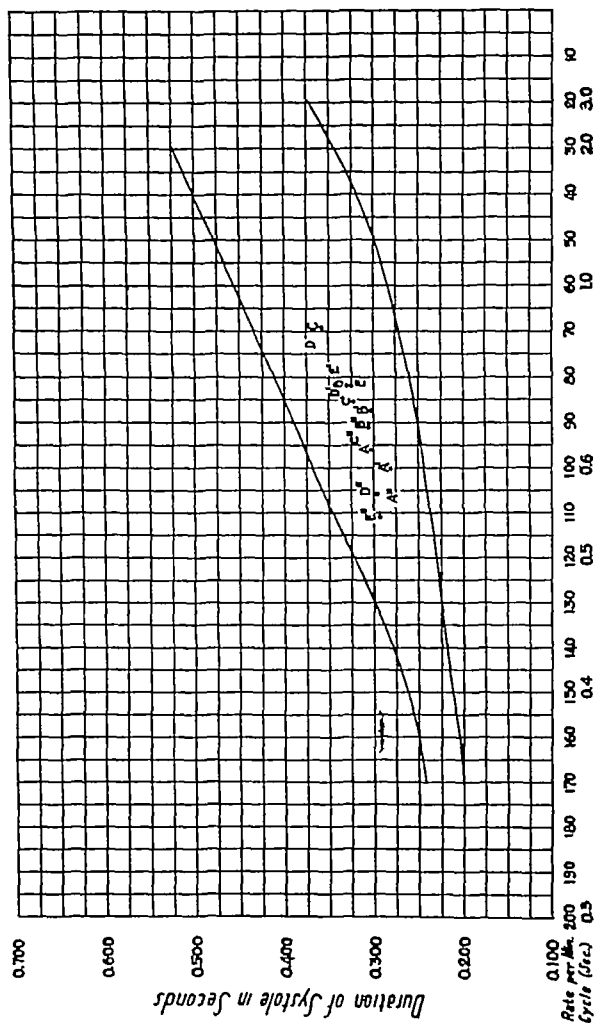
FIG 3 THE Q-T INTERVALS OF FIVE NORMAL SUBJECTS IN VARIOUS POSITIONS
A–E, in lying position, A'–E' in sitting position, and A"–E" in standing position
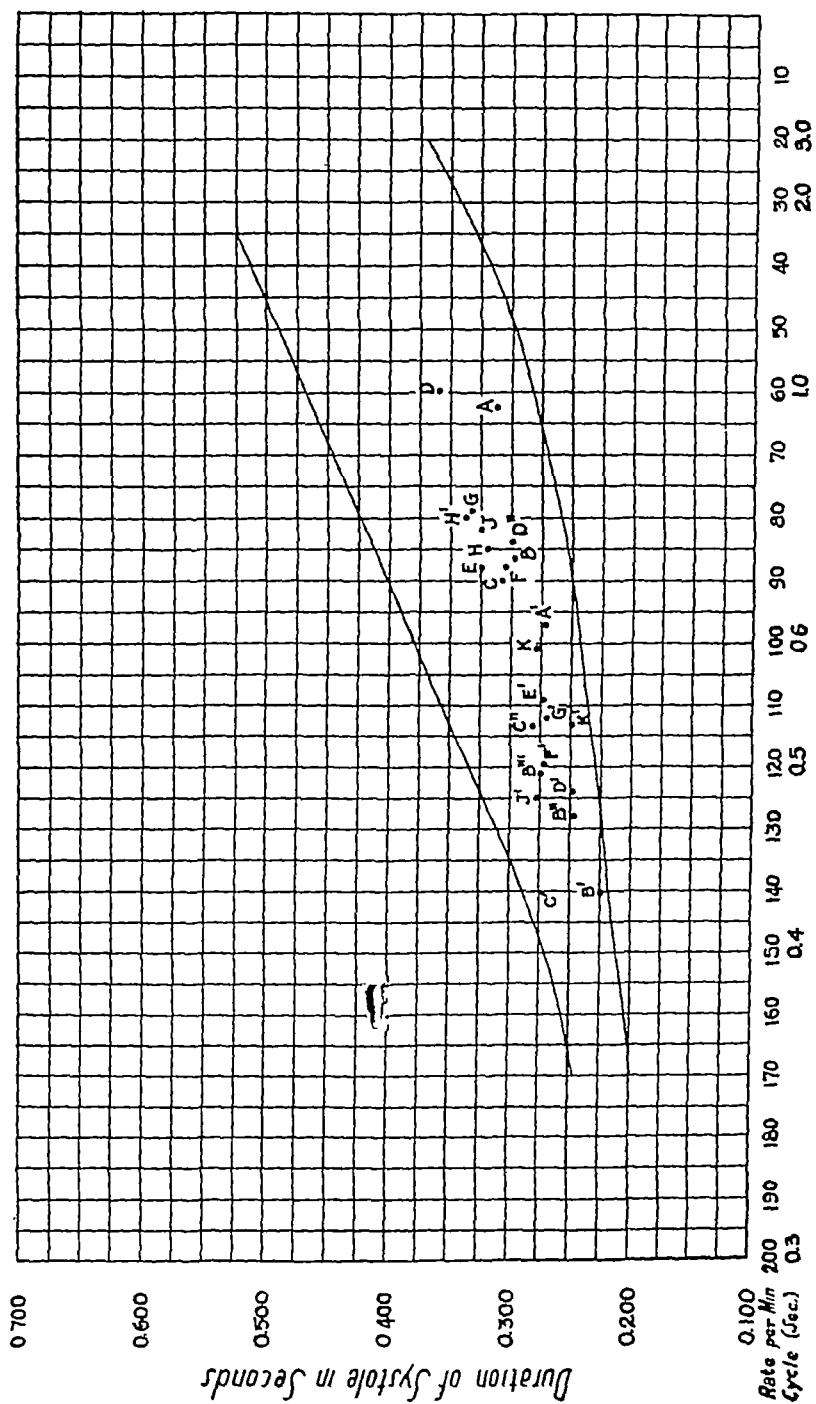
FIG 4 THE Q-T INTERVALS OF TEN NORMAL INDIVIDUALS BEFORE AND AFTER EXERCISE

A–K, at rest, A′–K′, B″–D″, B‴ after exercise
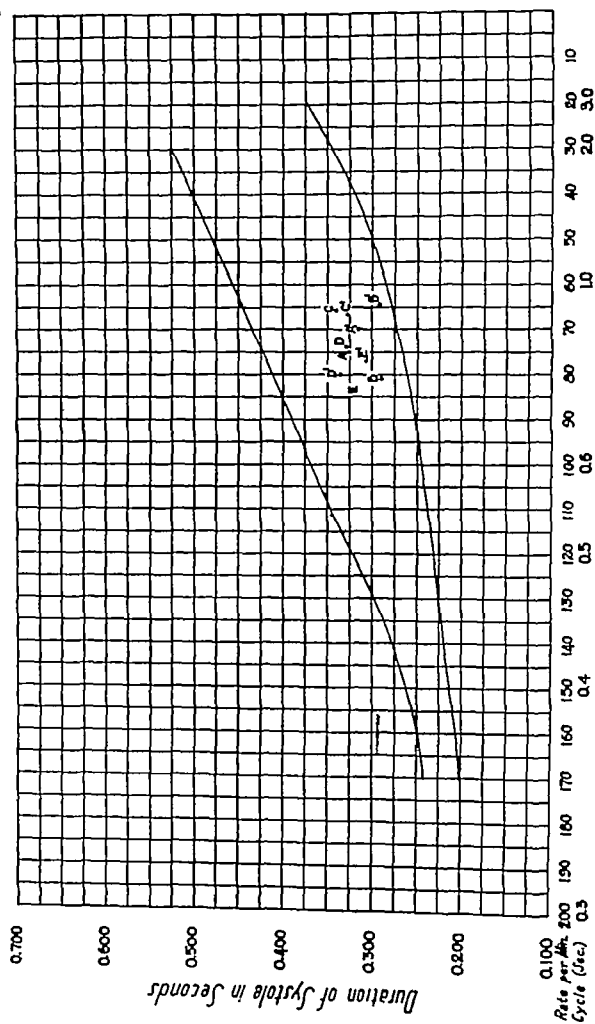
FIG 5 THE Q-T INTERVALS OF FIVE NORMAL INDIVIDUALS BEFORE AND AFTER DIGITALIZATION
A-E, before digitalis, A'-E' after digitalis

the variation in the duration of electrical systole   Heart rate alone as
in the records from the larger series of normal subjects in the sitting
position (fig 1) may be accounted the chief, though doubtless, not
the sole factor   Whatever part gravity may play in these five sub-
jects is a minor one   However, it is quite possible that heart rate
itself acts by its effect on the amount of blood entering the heart,
duration of electrical systole being controlled by this content of blood
rather than by the varying heart rate in itself   Other things being
equal, the faster the heart rate the less blood enters the heart, the
slower the rate the larger the blood volume in the ventricles   If this
is so it would appear that gravity too, with change in position, should
influence the duration of systole, but this influence is not apparent
in the measurements of the present five subjects

3 Normal cases after exercise   In figure 4 are charted the results
of exercise on the duration of ventricular systole in 10 normal subjects
(young men)   First, the subjects were electrocardiographed at rest
(A to K) and then immediately after exercise consisting of running up
and down four flights of stairs (A' to K')   Three subjects were
recorded a second time after exercise (B'', C'', D'') and one was
recorded a third time (B''')

It is obvious that all the measurements remained well within the
normal limits as charted from figures 1 and 2

4 The effect of digitalis on normal subjects   Five normal subjects
were digitalized (to mild toxic effects) in the course of 7 to 10 days
(2 0 to 3 0 grams of digitalis leaf by mouth), and electrocardiograms
before digitalis and at the height of the effect of the drug were meas-
ured   As will be seen in figure 5, all measurements were well within
normal limits permitting us to conclude that digitalis in the normal
subject does not influence appreciably the duration of electrical systole
as measured by the Q-T interval of the electrocardiogram

II Abnormal cases

We now turn to strictly pathological conditions and will consider
first structural defects such as cardiac enlargement and valvular
disease, then the effect of other conditions and diseases like hyper-
tension per se and diabetes, and finally functional conditions like
congestive failure and abnormal tachycardias, bradycardias, and
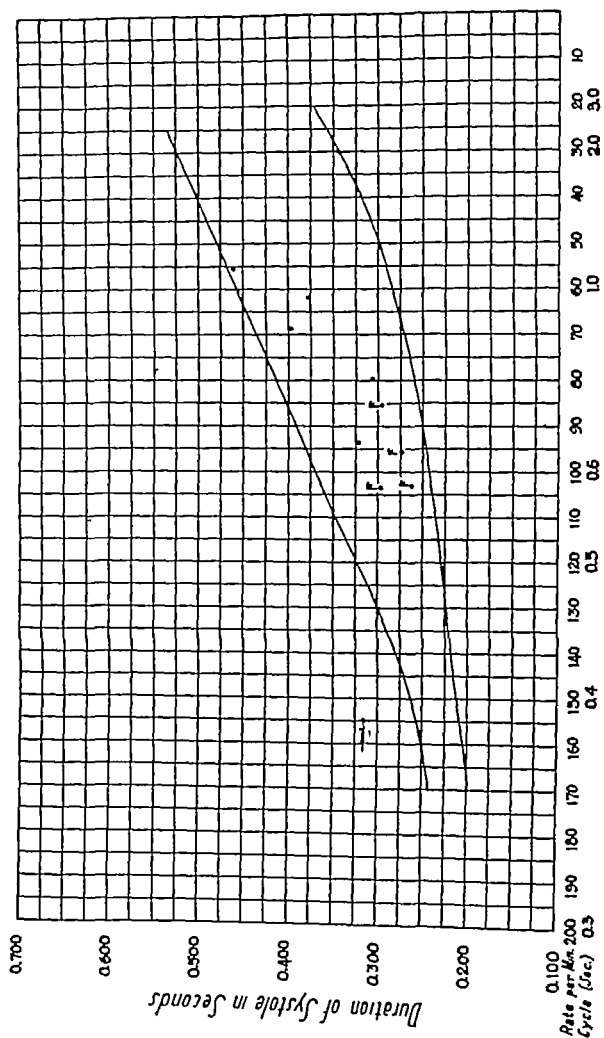
FIG 6 THE Q-T INTERVAL OF TEN PATIENTS WITH VERY LARGE HEARTS (WITHOUT ABNORMAL AXIS DEVIATION

F = four cases with auricular fibrillation also
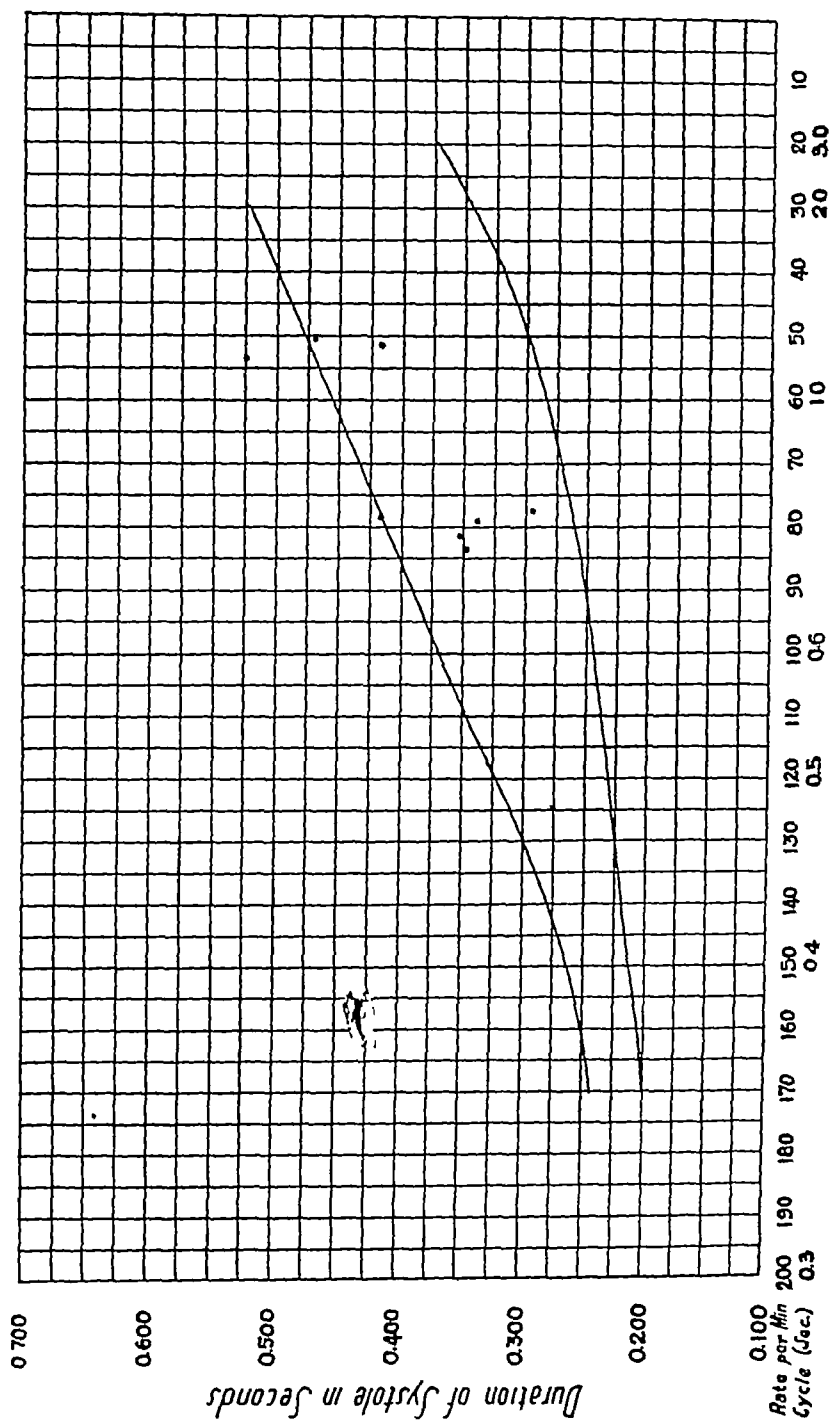
FIG 7 THE Q-T INTERVALS OF NINE PATIENTS WITH VERY LARGE HEARTS AND ABNORMAL LEFT AXIS DEVIATION

arrhythmia   There are included in these pathological groups a total of 163 cases

1 Structural   A Enlargement of the heart without abnormal axis deviation   Ten cases with marked cardiac enlargement but without abnormal axis deviation were studied   In some, the cause of the enlargement was unknown   In a few, hypertension or aortic regurgitation were factors   Six had normal rhythm and four had auricular fibrillation   The results are charted in figure 6 and the Q-T interval is within normal limits in every instance.

We had thought that marked enlargement of the heart might cause prolongation of electrical systole. This proved not to be the case when the Q T intervals of this group of cases were measured

B Enlargement of the heart with abnormal left axis deviation   To supplement the cases of cardiac enlargement without abnormal axis deviation, nine more cases with enlarged heart and an abnormal degree of left axis deviation were studied   Hypertension and aortic regurgitation were the chief underlying factors   Only one of these nine patients showed a Q-T interval longer than the normal outer limits (fig 7)   One other case was on the upper border line of normal and one just within it.   The others were well inside the normal limits

Thus of 19 cases with very large hearts, only one showed a duration of systole above the normal limits as measured by the Q-T interval It may be said, therefore, that cardiac enlargement does not ordinarily cause an increase in the Q-T interval

C Aortic regurgitation   Nine cases of aortic regurgitation, due to rheumatic or luetic etiology, were studied and are charted in figure 8 In all the duration of systole was normal

D Aortic stenosis   Aortic stenosis, both in animal experimentation and in the clinic, has been reported as a cause of systolic lengthening (13, 20, 37, and 38)   Figure 9 shows the results of the measurement of electrical systole of 6 cases of our own, one of which, very marked in degree, was confirmed by post mortem examination   All measurements fall within normal limits, including even the one case B with slight bundle branch block in addition

E Mitral stenosis   Ten cases of mitral stenosis all showed Q-T intervals well within normal limits (fig 10)

F Congenital heart disease   Eight cases of congenital cardiac
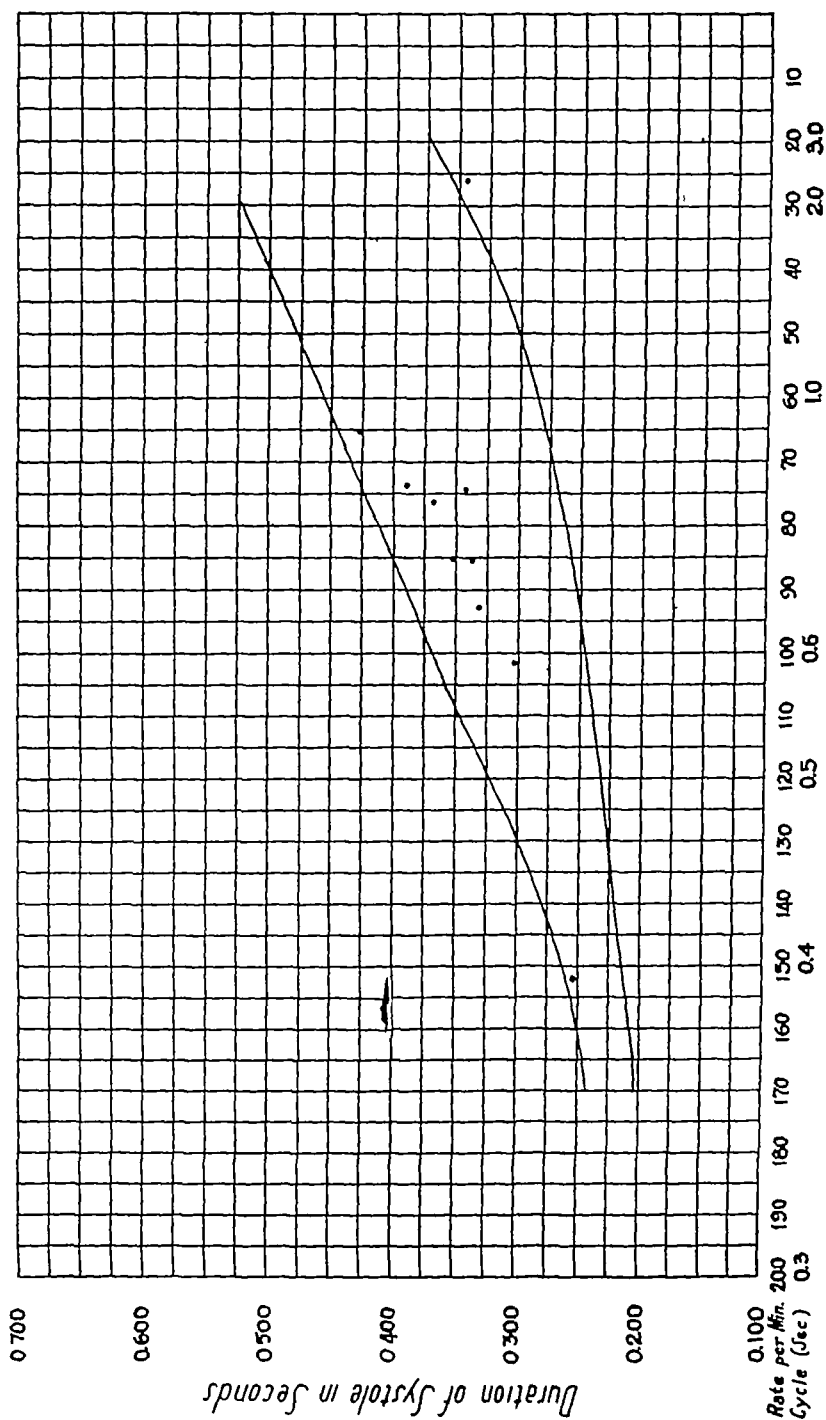
FIG 8 THE Q-T INTERVALS OF TEN PATIENTS WITH AORTIC REGURGITATION
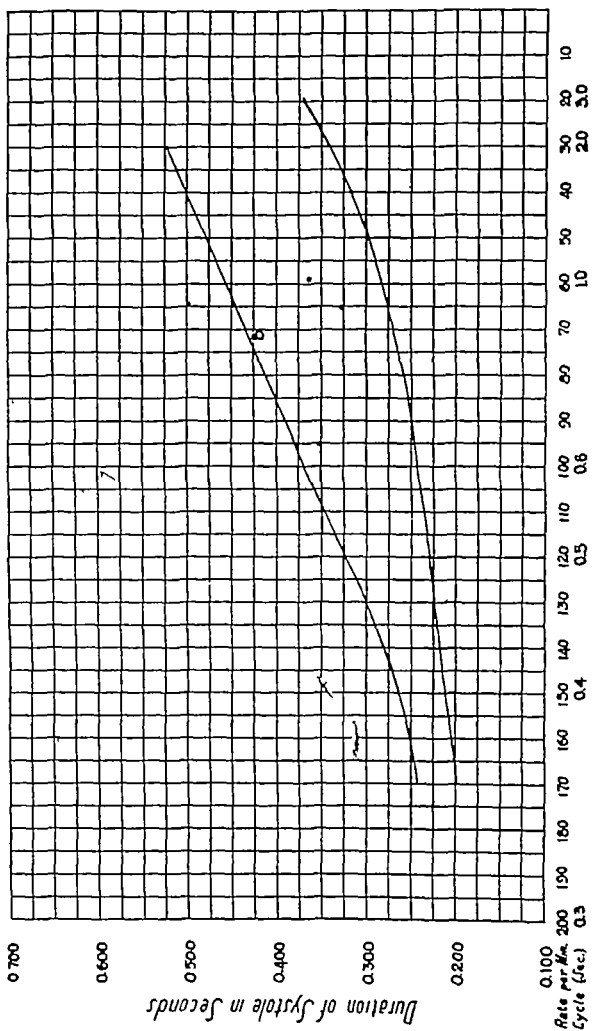
FIG 9 THE Q-T INTERVALS OF SIX PATIENTS WITH AORTIC STENOSIS, ONE CASE B HAD ALSO RIGHT BUNDLE BRANCH BLOCK
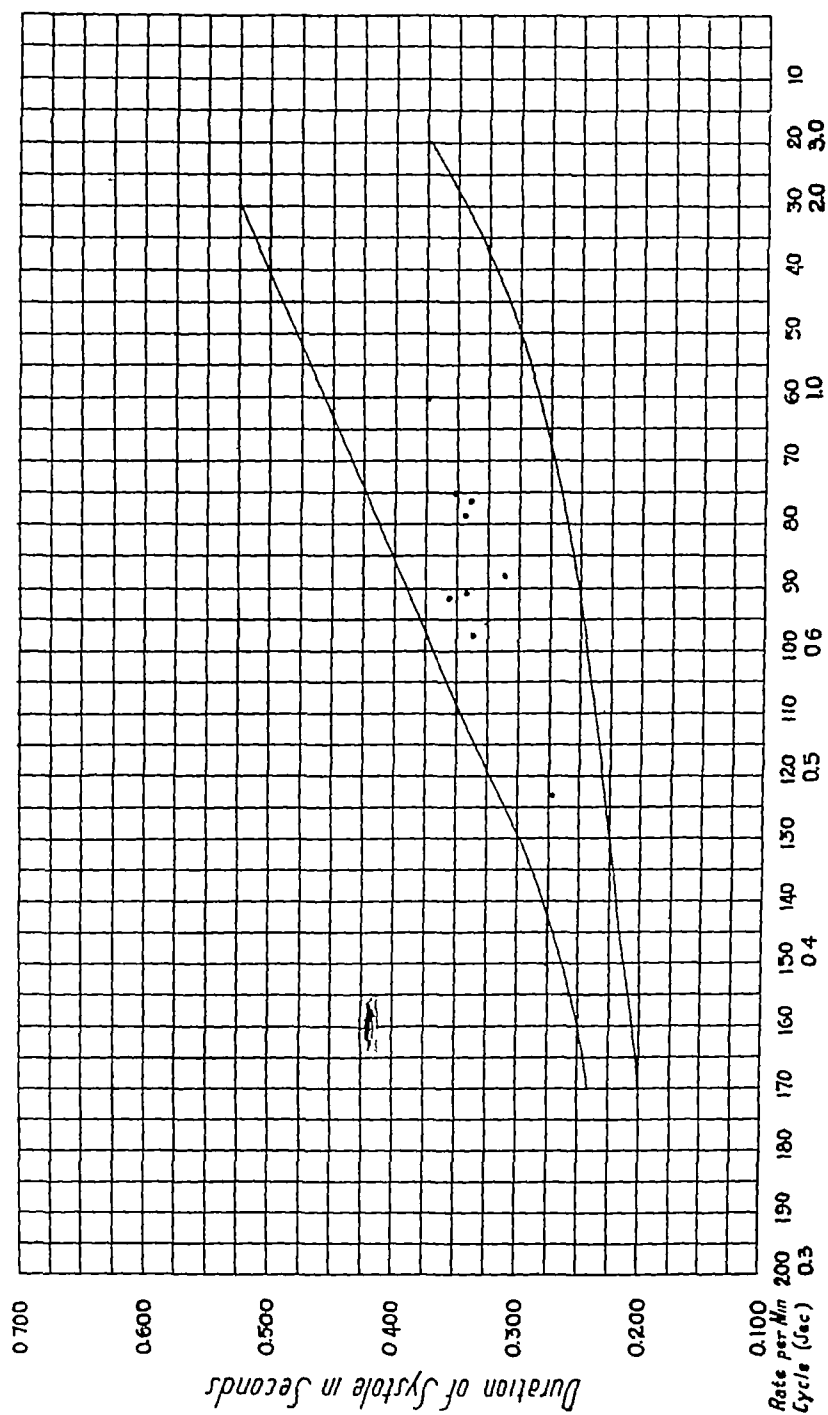
FIG 10 THE Q-T INTERVALS OF TEN PATIENTS WITH MITRAL STENOSIS
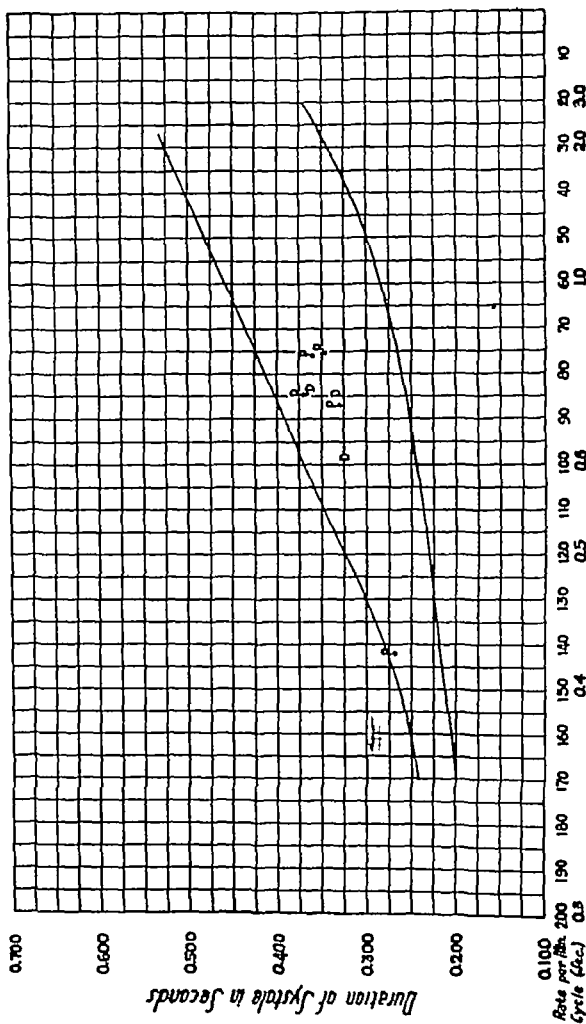
FIG 11 THE Q-T INTERVALS OF EIGHT PATIENTS WITH CONGENITAL HEART DISEASE
D = those with patent ductus arteriosus (3) P = those with pulmonic stenosis (5)
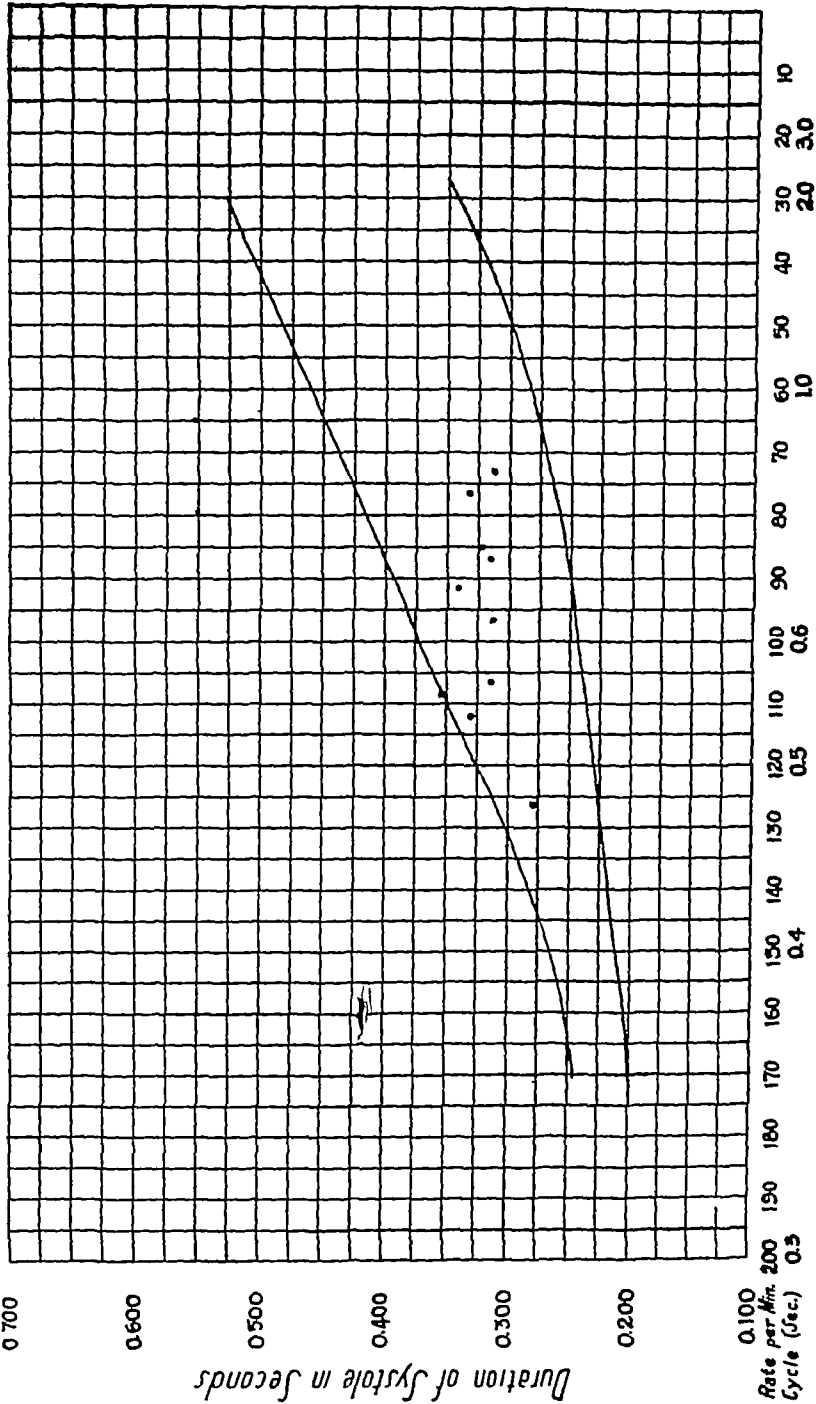
FIG 12 THE Q-T INTERVALS OF TEN CASES WITH UNCOMPLICATED ESSENTIAL HYPERTENSION
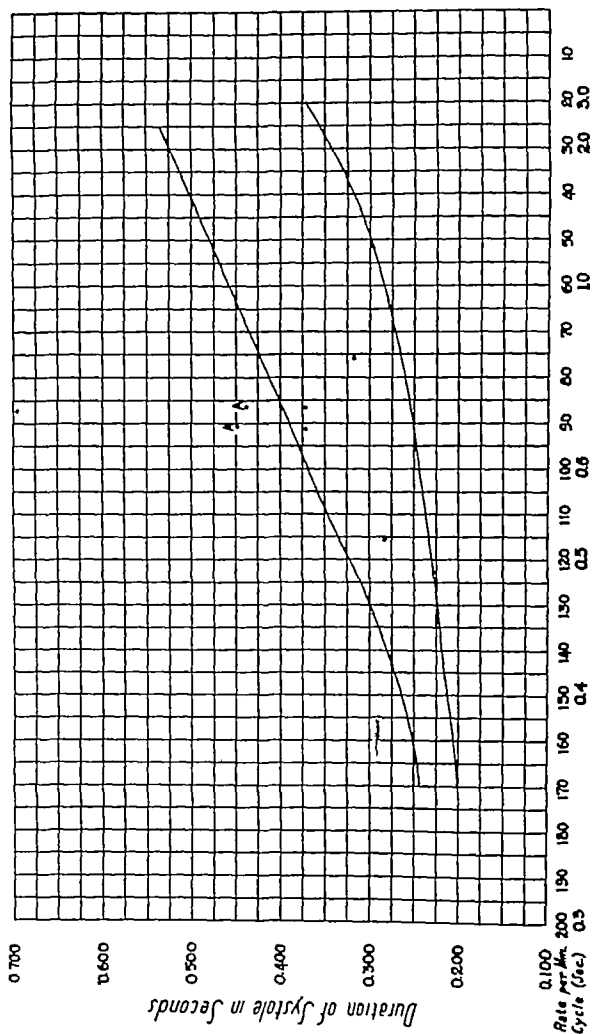
FIG 13 THE Q T INTERVALS OF FIVE CASES WITH UREMIA

A = 2 records of one case
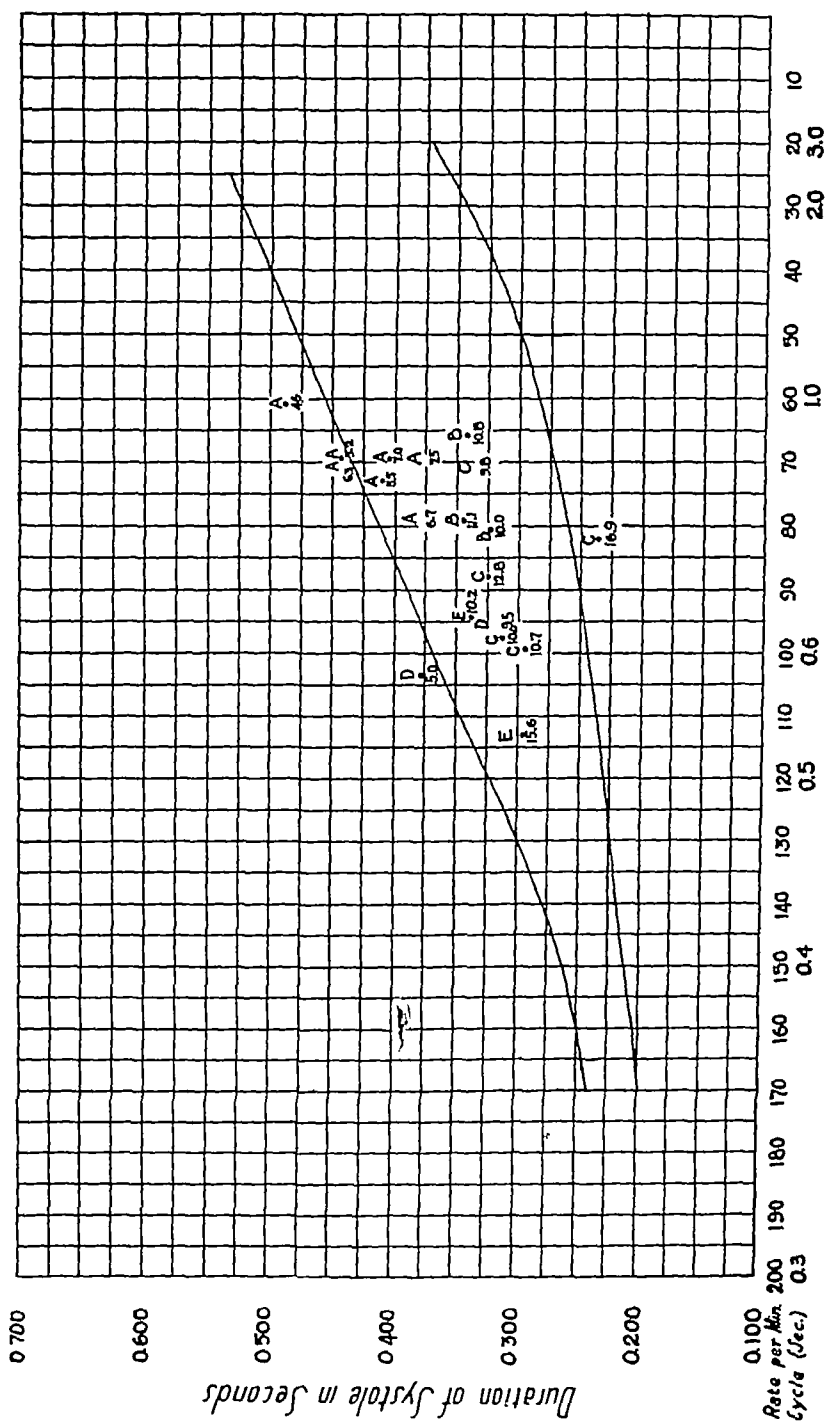
Fig 14 The Q-T Intervals of Five Patients A–E with Varying Blood Serum Calcium Content

The figures showing the amount of calcium (in milligrams per 100 cc serum) in the blood are given below each dot representing a Q-T interval
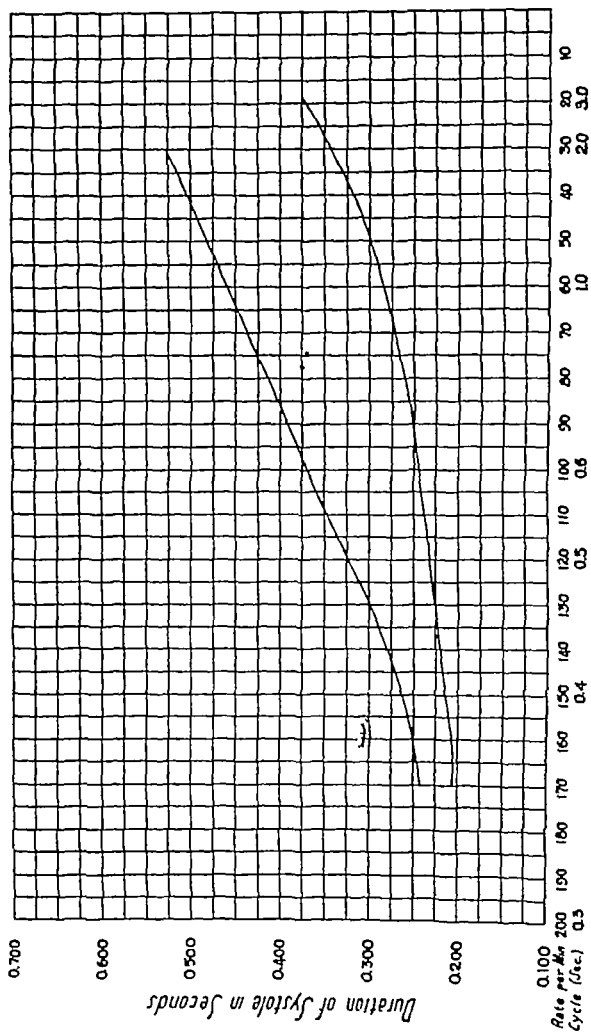
FIG 15 THE Q-T INTERVAL OF FIVE PATIENTS WITH DIABETES MELLITUS

defects all showed normal duration of systole as measured by the Q-T interval   Five of the patients were diagnosed as having pulmonic stenosis (P) and three as having patent ductus arteriosus (D)

2   Extrinsic factors   A   Hypertension   Hypertension has at times past been found present when systole is prolonged (28, 35 and 37) but in ten cases that we have studied in which the single factor of hypertension entered, we found all with a Q-T interval within normal limits, though one was on the upper limit of normal   These cases showed little or no cardiac enlargement by physical examination or x-ray and no abnormal axis deviation by electrocardiogram

B   Uremia   Five cases of uremia showed normal duration of electrical systole in all except one (fig 13)   On both of two separate occasions, this patient's Q-T interval was too long

C   Blood calcium studies   As shown by figure 14, the content of calcium in the blood serum appears definitely to be a factor in controlling the duration of the Q-T interval, in almost as striking a manner as is the pulse rate   This relationship has already been pointed out by Carter and Andrus (34) and our findings are strongly confirmatory   Five cases have been studied by us from the standpoint of calcium content of the blood serum and the two cases (tetany) which showed marked diminution of the serum calcium (A with 4 0 mgm and D with 5 0 mgm per 100 cc of blood) both showed prolongation of the Q-T interval beyond the normal, with a return to normal as the blood calcium rose

D   Diabetes mellitus   Five cases of diabetes mellitus were studied by us, three of them during the stage of hyperglycemia, all showed Q-T intervals within normal limits (fig 15)

E   Hyperthyroidism   The effect of hyperthyroidism was studied in eleven patients, in two of whom electrocardiograms were taken before and after correction of the hyperthyroidism by operation Also in two of the cases, records were obtained during periods of auricular fibrillation as well as during normal rhythm   As will be seen in figure 16, all the measurements are within normal limits except two, one a case with a metabolic rate of plus 32 per cent and normal rhythm and the other after operation with a metabolic rate of −5 per cent and auricular fibrillation   This latter case showed during normal rhythm, normal Q-T intervals both before and after operation All the other cases showed normal duration of electrical systole
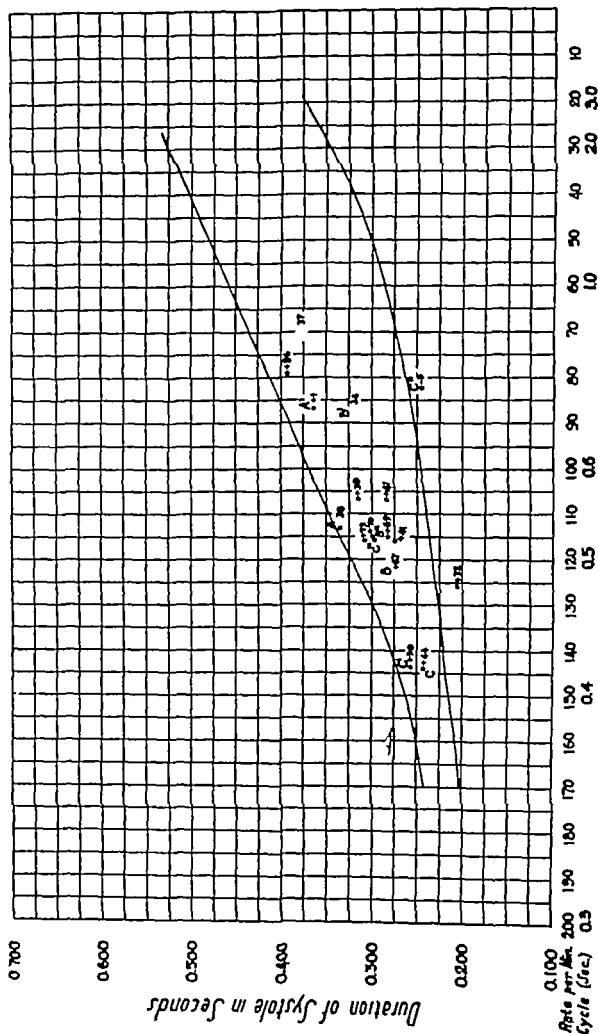
Fig 16 The Q-T Intervals of Eleven Patients with Hyperthyrodism, in Two of These Cases A and C the Q T Intervals After Operation and Correction of the Hyperthyrodism Are Also Shown (A', C'', C''') B'', C'' = cases during auricular fibrillation The figures below the dots give the basal metabolic rates
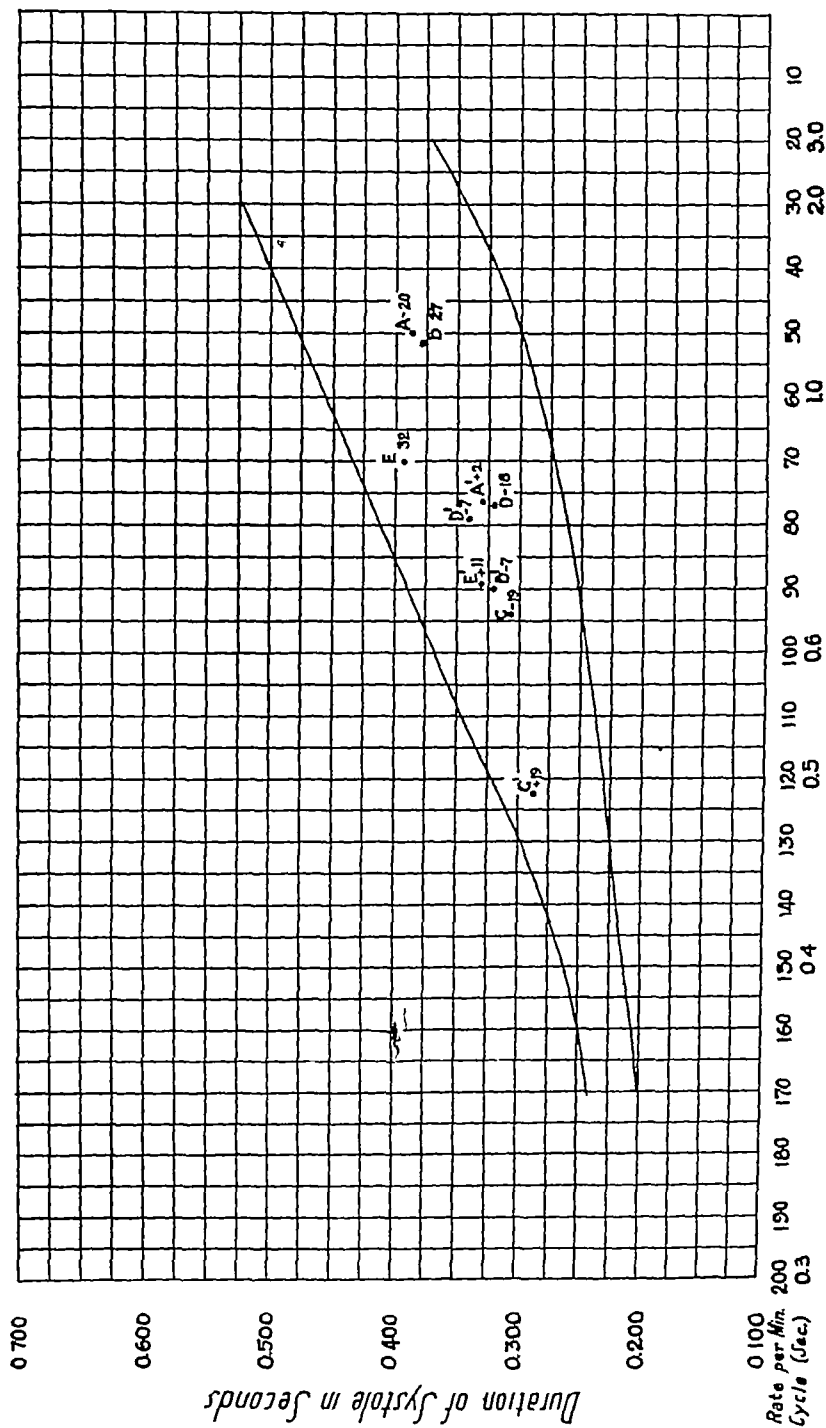
FIG 17 THE Q-T INTERVALS OF FIVE CASES WITH HYPOTHYROIDISM A–E   The basal metabolic rates are shown by figures below the dots representing the Q-T intervals The Q-T intervals of the same cases after thyroid therapy are also given (A′–E′)
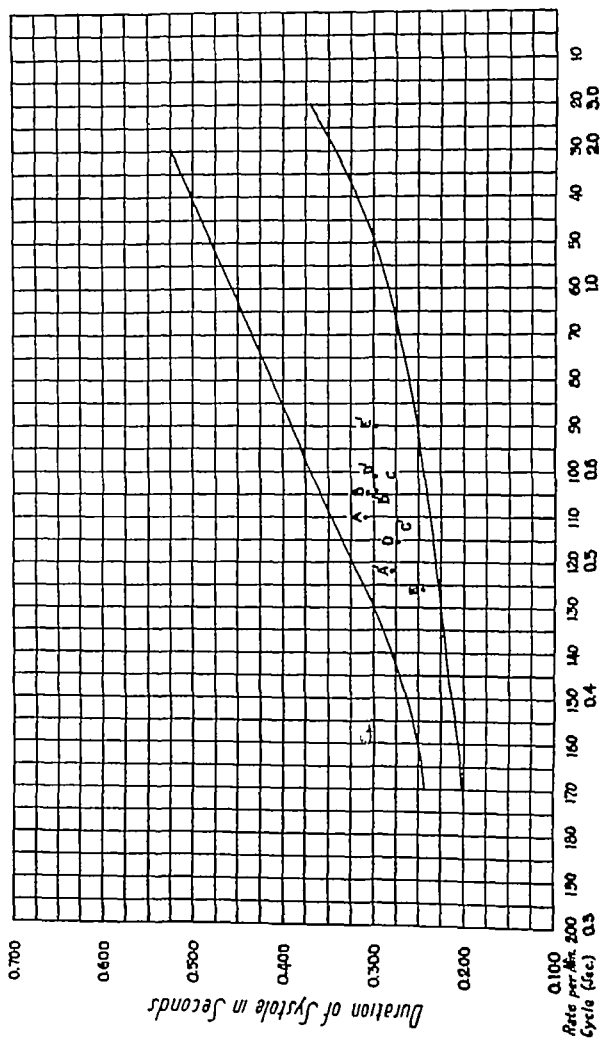
FIG 18 THE Q-T INTERVALS OF FIVE PATIENTS DURING (A-E) AND AFTER (A'-E') ACUTE INFECTION
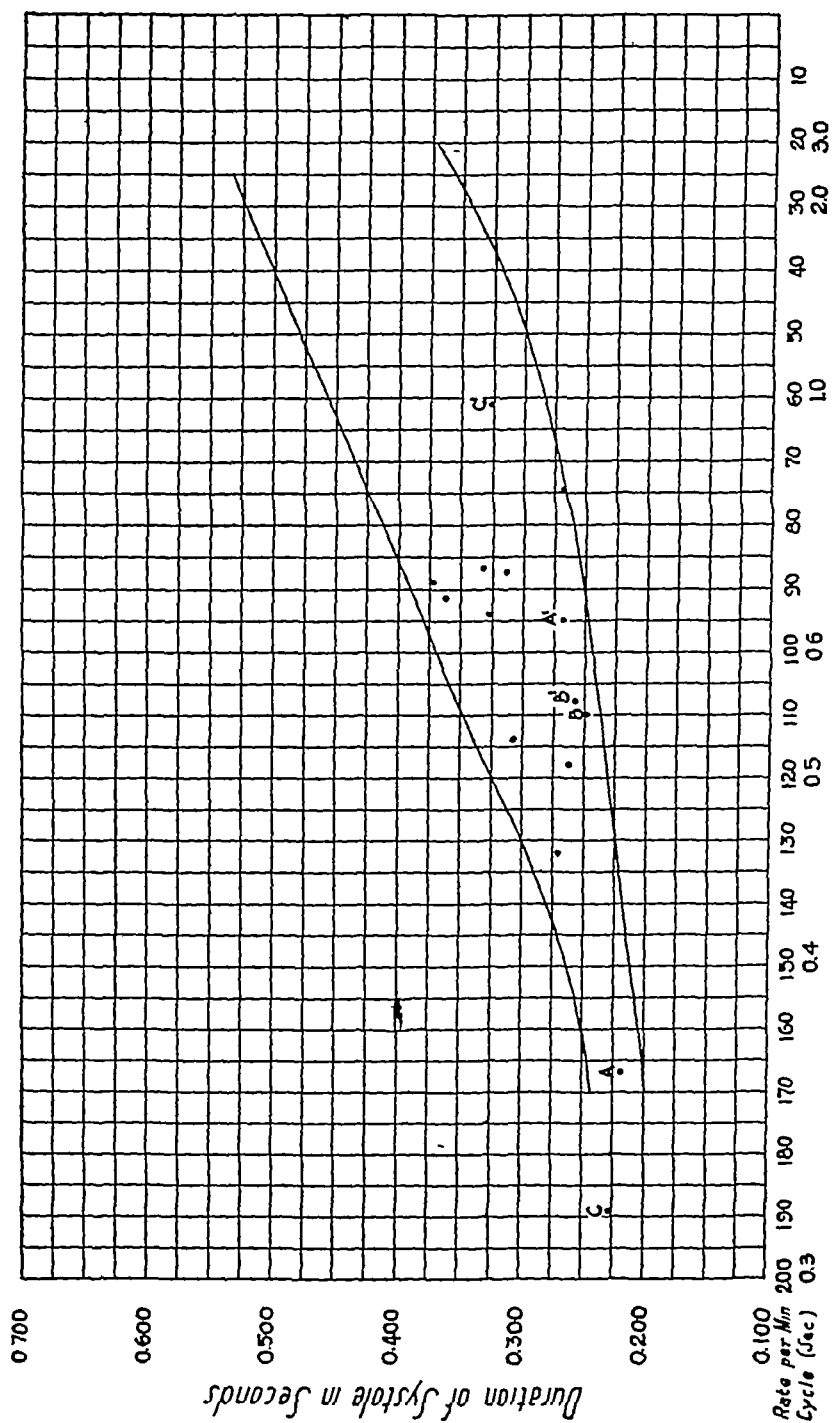
FIG 19   THE Q-T INTERVALS OF TWELVE PATIENTS WITH CONGESTIVE FAILURE, IN THREE CASES (A, B AND C), THE Q-T INTERVALS AFTER RECOVERY ARE ALSO SHOWN (A', B' and C')

Auricular fibrillation additionally present in cases A and C

F Hypothyroidism The effect of hypothyroidism on the duration of the Q T interval was measured in five cases both before and after thyroid therapy   In every case, the findings were well within normal limits as seen in figure 17, although we had thought that a sluggish heart in myxedema might prolong electrical systole   Of course, the pulse rate was invariably slower during the hypothyroid stage, and in accord with the slower pulse rate, the Q-T interval was longer

G Acute infection Five cases have been studied to determine whether or not acute infection affects the duration of the Q-T interval Electrocardiograms were taken and measured both during the infection and after recovery   Pneumonia was the infection in two cases and tonsillitis and pharyngitis in the other three   The Q T interval during the infection was generally short but in exact relationship to the degree of tachycardia and hence well within normal limits

It is of considerable interest to observe that in two of the cases, the pulse rate was actually higher after the infection than during it, due to excitement and effort syndrome but nevertheless the Q T intervals varied consistently as the heart rate

3 Functional conditions   A Congestive failure To determine whether congestive failure affects the duration of electrical systole we studied twelve cases, three with auricular fibrillation and nine with normal rhythm   Three of these patients we investigated again after the congestive failure had disappeared under rest and digitalis We have already noted that digitalis per se does not affect the Q-T interval   All the measurements both during and after congestive failure were within normal limits as shown in figure 19

Heart muscle weakness and congestive failure have been mentioned in the literature as possible causes for prolongation of systole (2, 29) and also for shortening of systole (32, 37)   Our study of the twelve cases reported above shows no definite effect either one way or the other

B Auricular paroxysmal tachycardia Five cases of auricular paroxysmal tachycardia have been studied by us both during the paroxysms and also during normal rhythm   A little difficulty has been experienced at times in making the measurements but only those electrocardiograms allowing a reasonable degree of accuracy have been selected   As seen in figure 20, there is a tendency for the Q-T
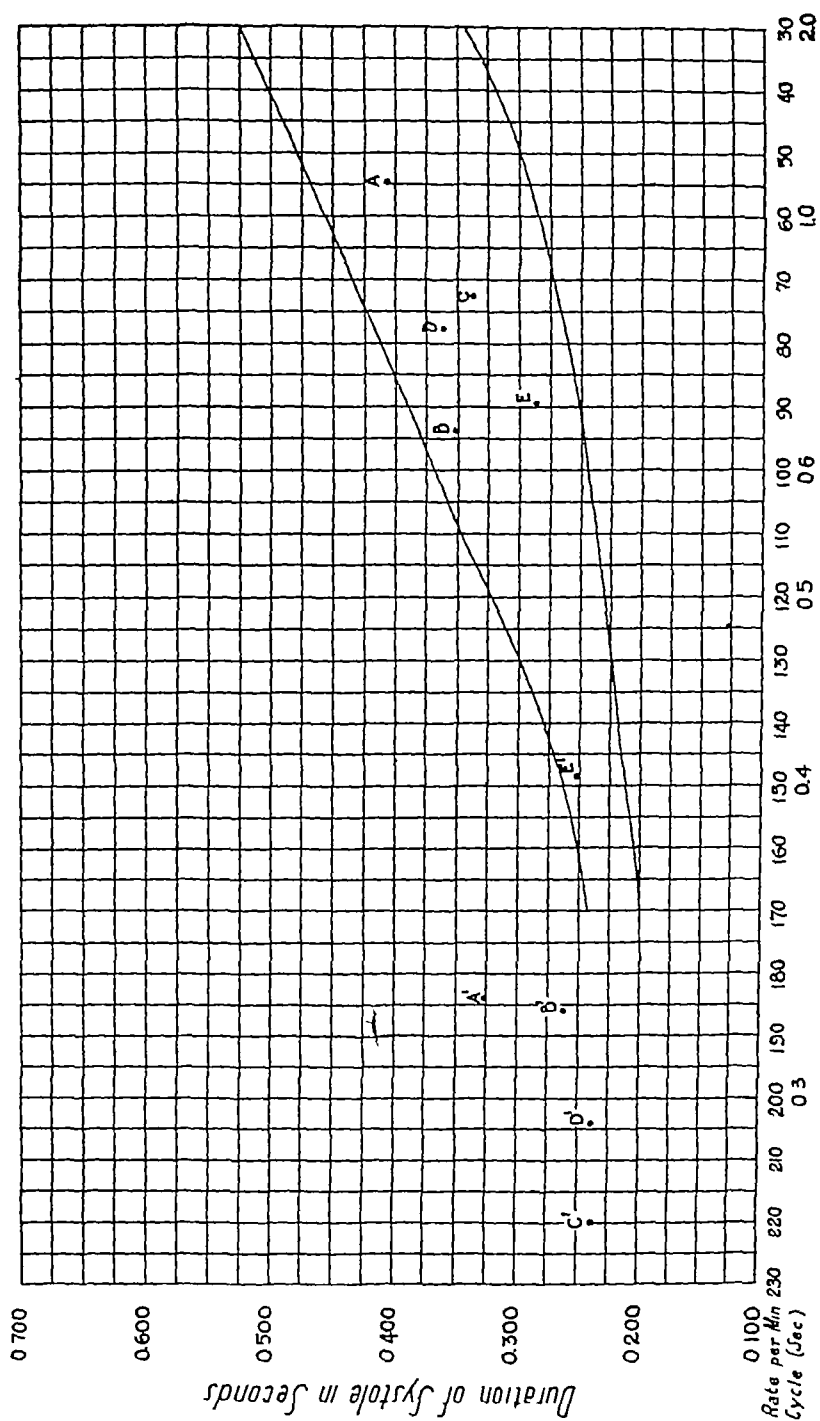
FIG 20 THE Q-T INTERVALS OF FIVE PATIENTS DURING AURICULAR PAROXYSMAL TACHYCARDIA (A'-E') AND DURING NORMAL RHYTHM (A-E)
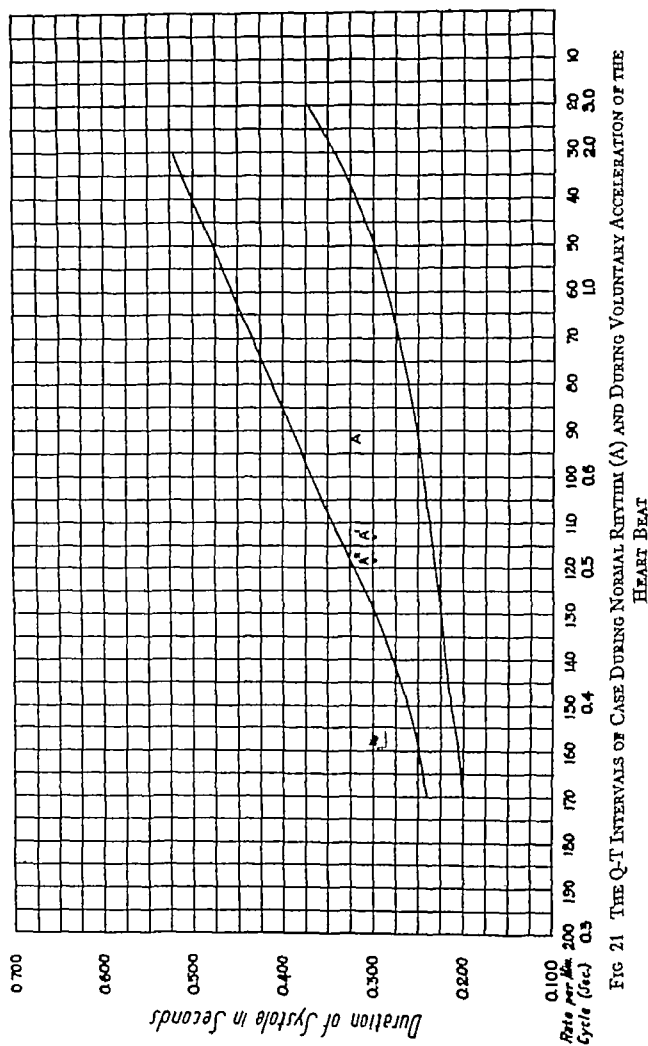
FIG 21 THE Q-T INTERVALS OF CASE DURING NORMAL RHYTHM (A) AND DURING VOLUNTARY ACCELERATION OF THE HEART BEAT
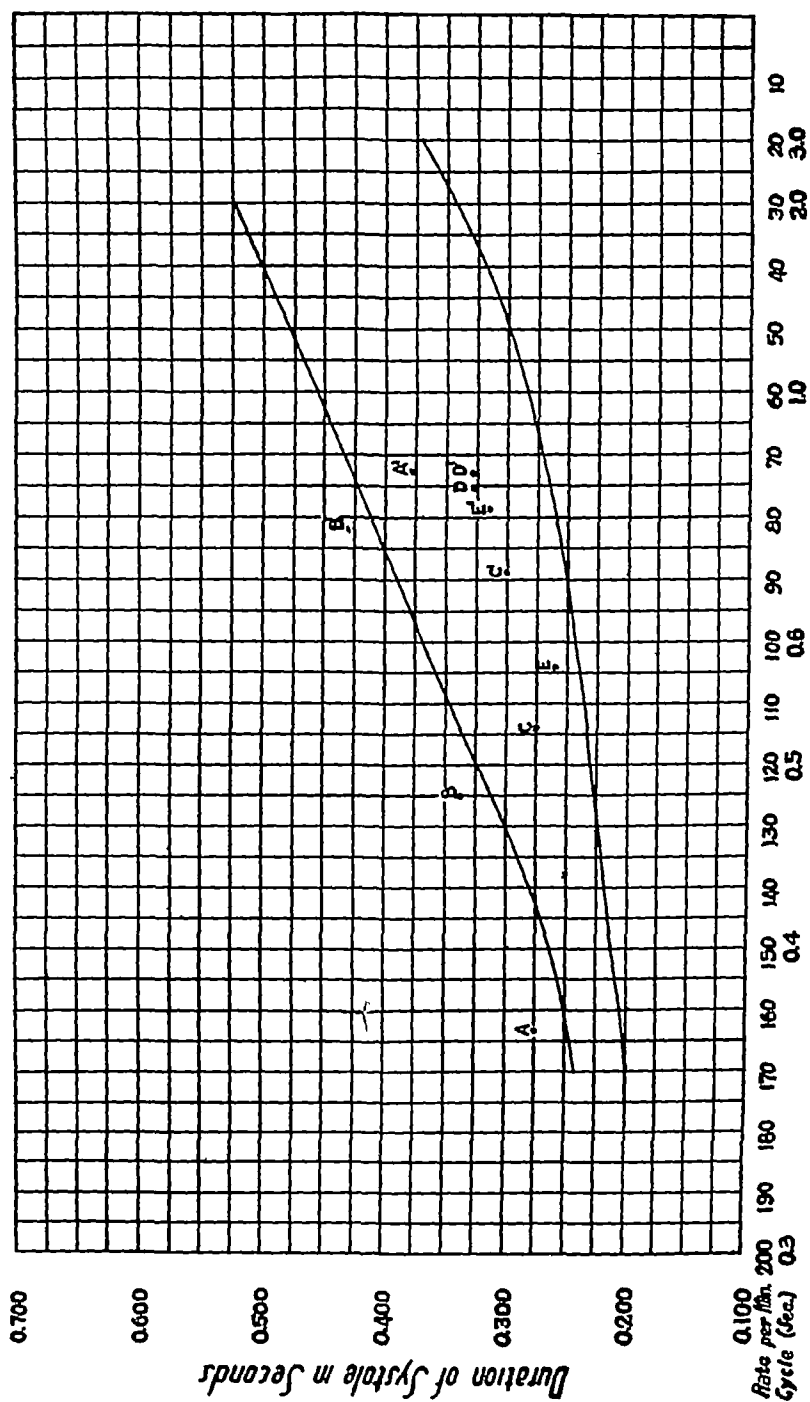
FIG 22   THE Q-T INTERVALS OF FIVE PATIENTS WITH AURICULAR FIBRILLATION (A–E) AND OF THE SAME PATIENT AFTER RESTORATION TO NORMAL RHYTHM BY QUINIDINE SULPHATE (A'–E')
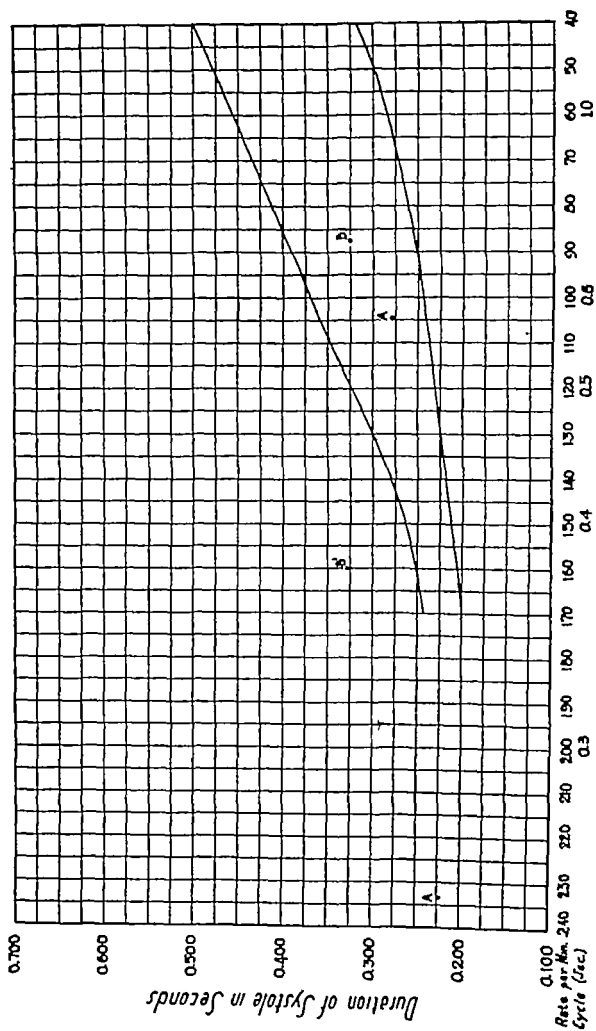
FIG 23 THE Q-T INTERVALS OF TWO CASES OF VENTRICULAR PAROXYSMAL TACHYCARDIA DURING THE PAROXYSM
(A', B') AND DURING NORMAL RHYTHM (A, B)

interval to be longer at the paroxysmal rates than would be expected on continuing the so-called normal limit curves   The slowest paroxysmal rate—148—was found with a Q-T interval within normal limits, but all four cases with rates over 180 showed relatively long Q-T intervals   Of course, here we have no normal controls for comparison

C   Voluntary acceleration of the heart rate   One rare case of voluntary acceleration of the heart rate was studied and found to have durations of the Q-T interval perfectly normal at varying rates (fig 21)

D   Auricular fibrillation with restoration of normal rhythm   The effect of auricular fibrillation on the duration of the Q-T interval was studied in five cases in whom normal rhythm was later restored by quinidine sulphate   A comparison of the Q-T interval during auricular fibrillation and during normal rhythm was made   The results are charted in figure 22   All measurements were normal except three which were prolonged, two of these measurements were from the same case (B B') during auricular fibrillation and during normal rhythm and that case had also right bundle branch block which will be discussed later, the third measurement was also delayed beyond the normal but occurred in a case only during a very rapid ventricular rate (164) in auricular fibrillation, the Q-T interval being within normal limits when the rhythm was normal and the rate slow (73)   The other three cases showed entirely normal rhythm   It would seem then from these measurements and those of seven other cases with auricular fibrillation shown in figures 6 and 19, that auricular fibrillation in itself does not affect the Q-T interval, which may be prolonged, however, if there is a coincident bundle branch block or an extreme tachycardia   Auricular fibrillation has been reported in some cases in the literature with overlong Q-T intervals (2) and also with Q-T intervals shorter than normal (2, 31, 32, 36)

E   Ventricular paroxysmal tachycardia   Two cases of ventricular paroxysmal tachycardia were electrocardiographed during their paroxysms and during normal rhythm   The results are shown in figure 23   In one case the Q-T interval is much prolonged beyond the normal outer limit (B') and in the other case the Q-T interval falls also above this upper limit line if projected, but only a little above normal   In both cases the Q-T interval is well within normal limits during normal rhythm
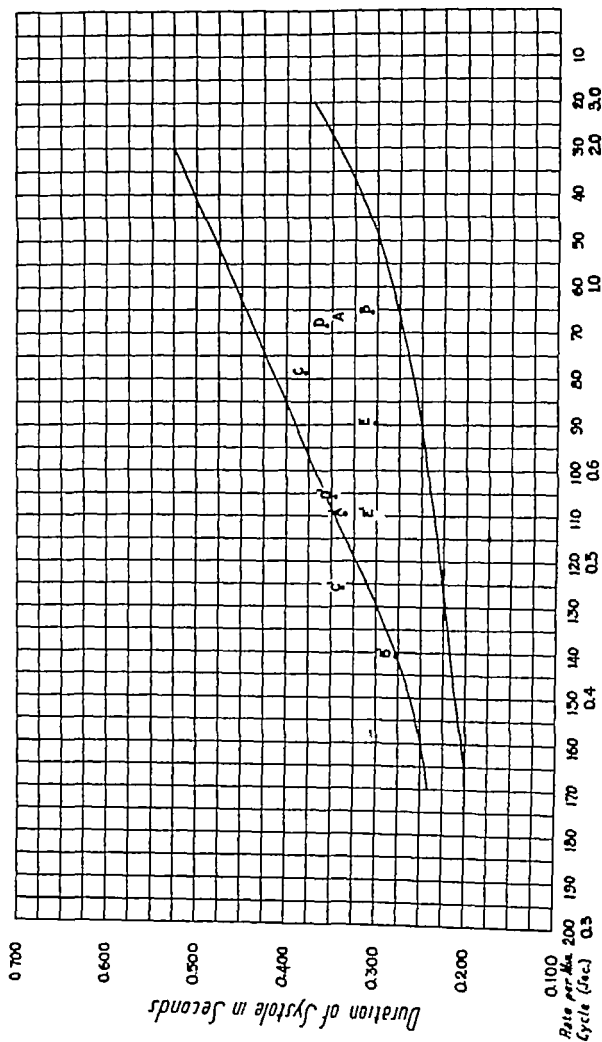
Fig 24 The Q-T Intervals of Auricular Premature Beats (A'-E') and of Normal Beats (A-E) in Five Cases
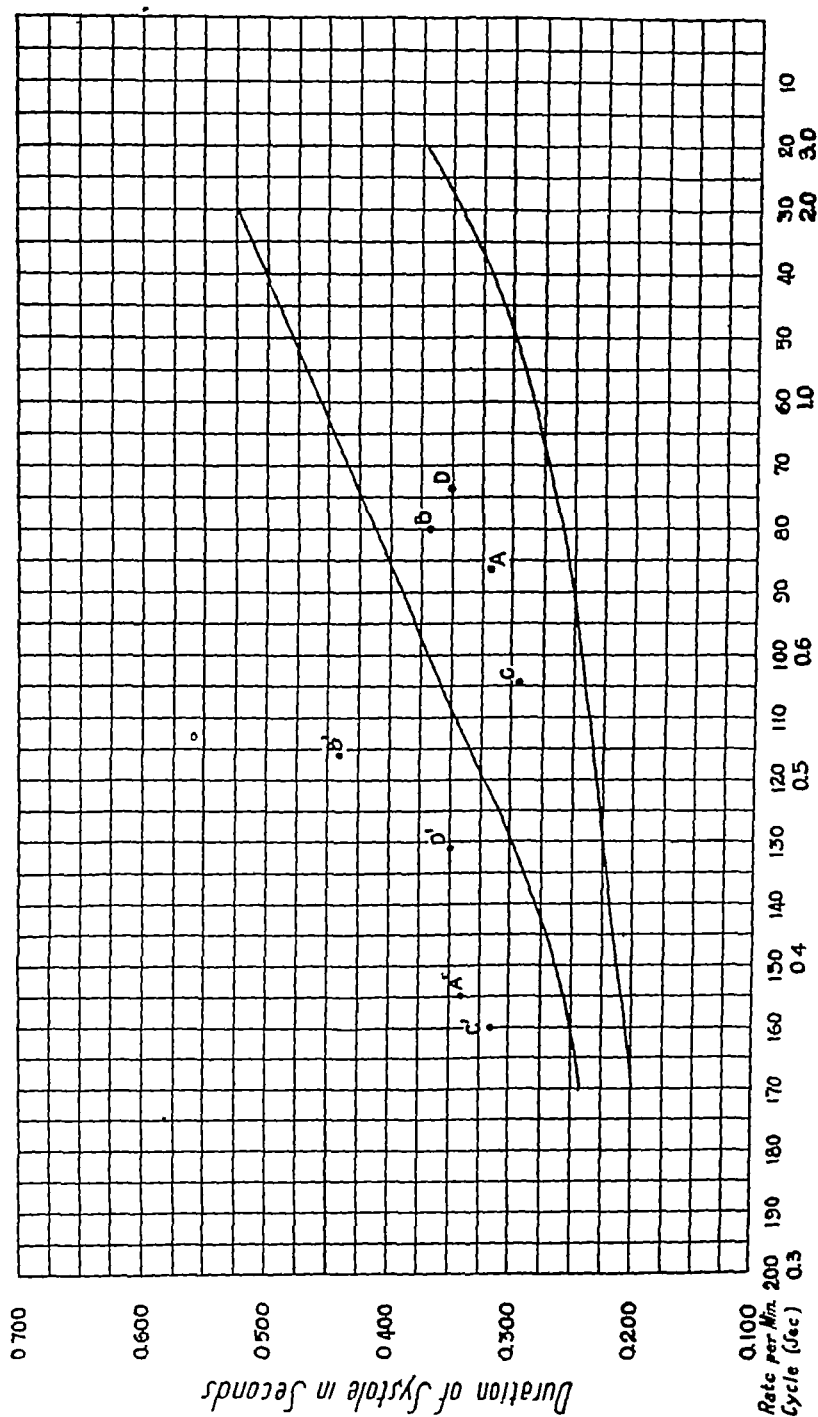
FIG 25   THE Q-T INTERVALS OF VENTRICULAR PREMATURE BEATS (A′–D′) AND OF NORMAL BEATS (A–D) IN FOUR CASES
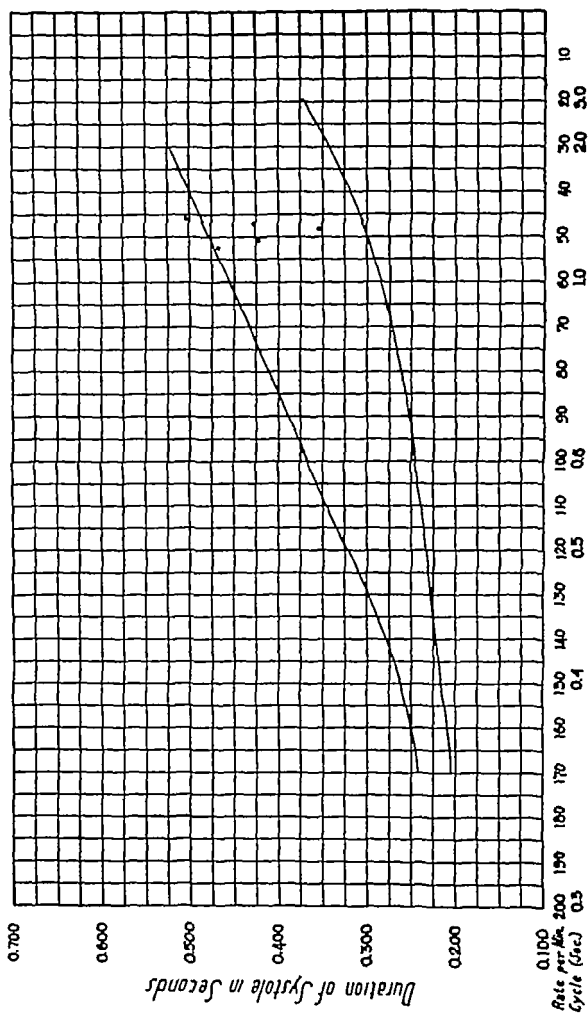
Fig 26 The Q-T Intervals of Seven Cases of Sinoauricular Bradycardia
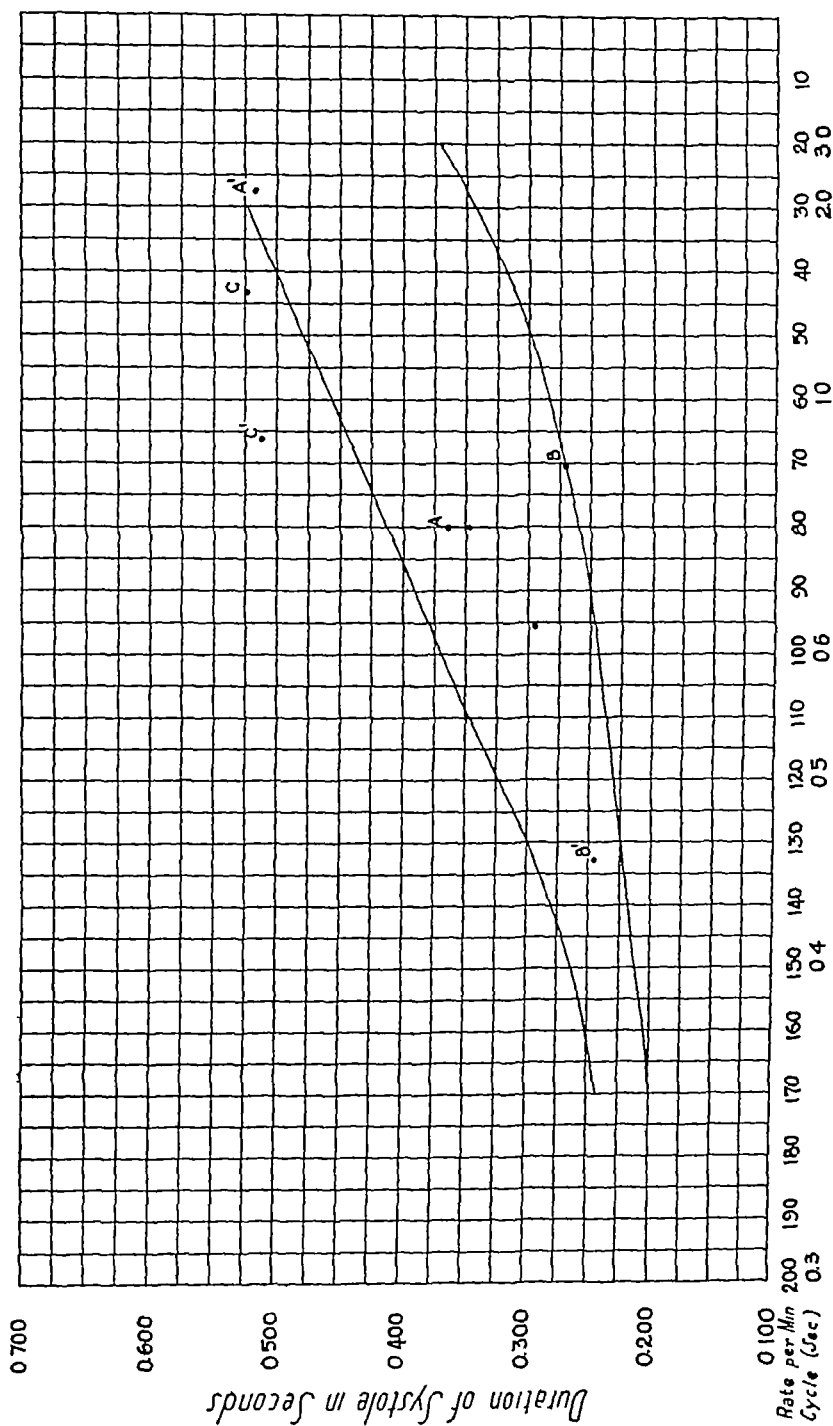
FIG. 27 THE Q-T INTERVALS OF FIVE PATIENTS WITH PARTIAL AURICULO-VENTRICULAR BLOCK

A–A', B–B', and C–C', represent the Q-T intervals in three of the cases at varying degrees of block and heart rate

F Auricular premature beats A comparison has been made in five cases between the duration of the Q-T interval of an auricular premature beat without aberration and that of a normal beat (fig 24) In one case the auricular premature beat Q-T interval was beyond the upper normal limit and in three other cases on or very close to it Only one was well within normal limits The "rate" for the premature beats was calculated from the interval between the preceding normal beat and the premature beat

G Ventricular premature beats A similar study was made of four cases of ventricular premature beats Figure 25 shows that in all of the four cases the Q-T interval of the premature beat was much prolonged beyond the normal limits for heart rates based on the interval between the preceding normal beat and the premature beat This is what one would expect since the abnormal origin of the beat should cause a slower spread of the impulse and slower rise of intraventricular pressure than normally

H Sinoauricular bradycardia Seven instances of sinoauricular bradycardia without heart disease were studied All Q T intervals were within normal limits except one which was slightly overlong (fig 26)

I Partial auriculo ventricular block Five cases of partial auriculo-ventricular block have been examined and are charted in figure 27 One was electrocardiographed during a period of high grade partial block at two different rates in each case All measurements of electrical systole except the two of one of the cases are within normal limits though varying rather widely The one case with prolonged Q-T interval during both fast and slow rates with block was a boy of six years with miliary tuberculosis

J Complete auriculo ventricular block Six cases of complete auriculo-ventricular block showed abnormally long Q-T intervals in all except two and those two were close to the upper edge of normal However, three of these four case with overlong Q T intervals had also bundle branch block and the Q-T intervals were extraordinarily prolonged, with one exception the longest that we have encountered (all over 0 6 second in duration) One of them with varying bundle branch block gave a measurement of the Q-T interval of over 0 7 second, the longest of our entire series and about 0 2 second above
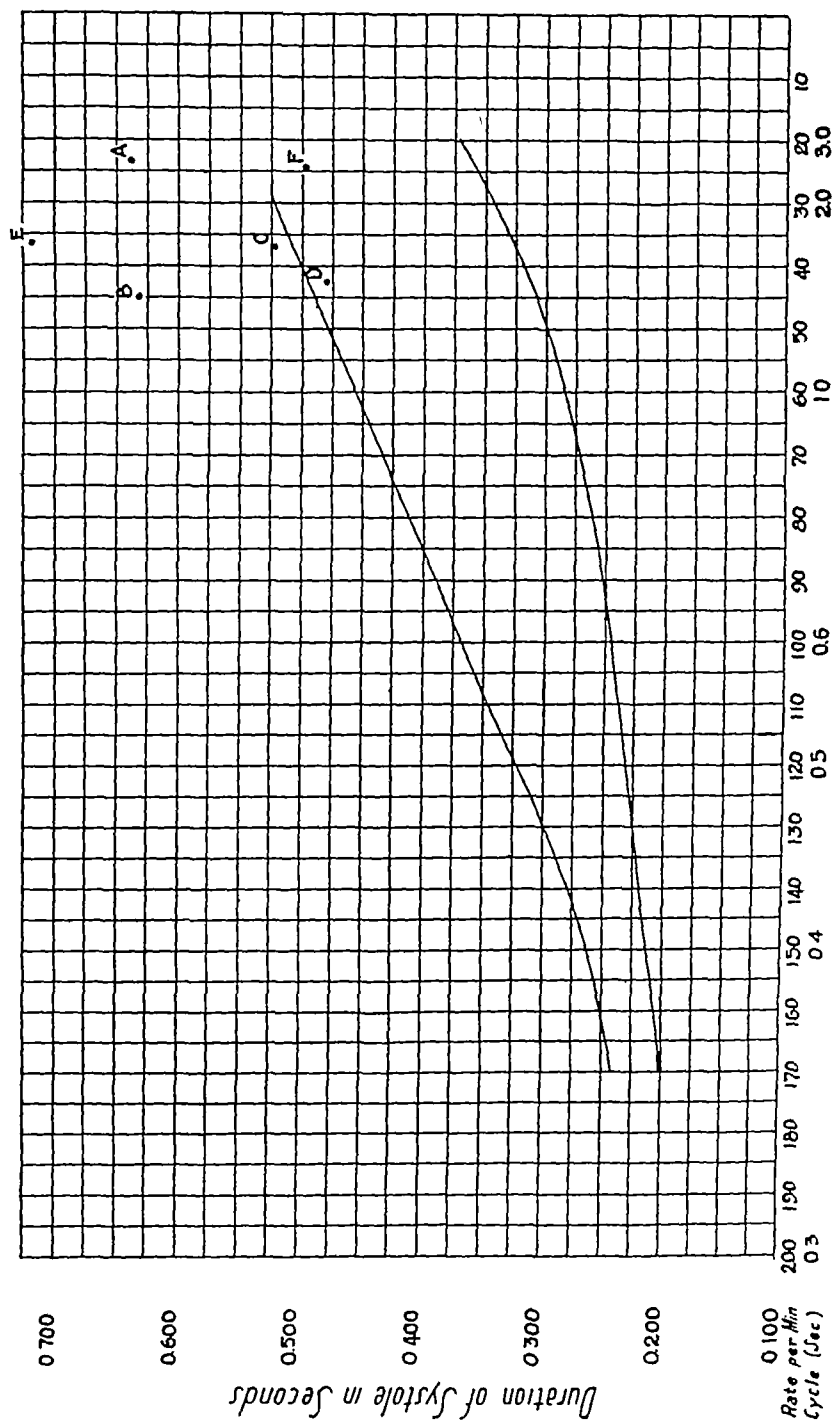
FIG. 28 THE Q-T INTERVALS OF SIX PATIENTS WITH COMPLETE AURICULO-VENTRICULAR BLOCK

Cases A, B and E had also bundle branch block
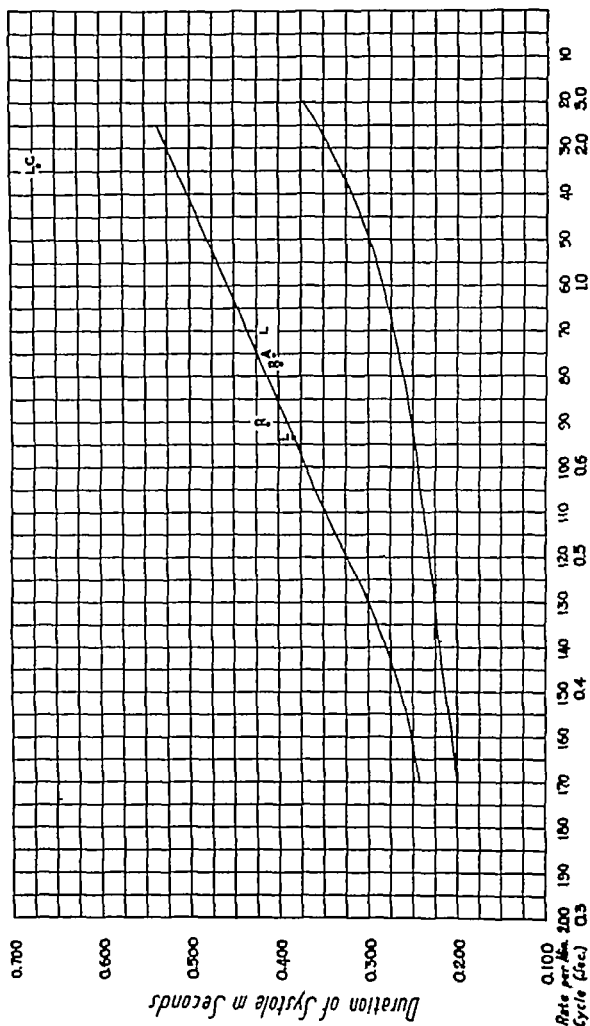
Fig 29 The Q-T Intervals of Six Patients with Bundle Branch Block

Cases I, L had left bundle branch block, case LC left bundle branch block and complete auriculo-ventricular block, cases R, R right bundle branch block, and case A intraventricular block of lesser grade
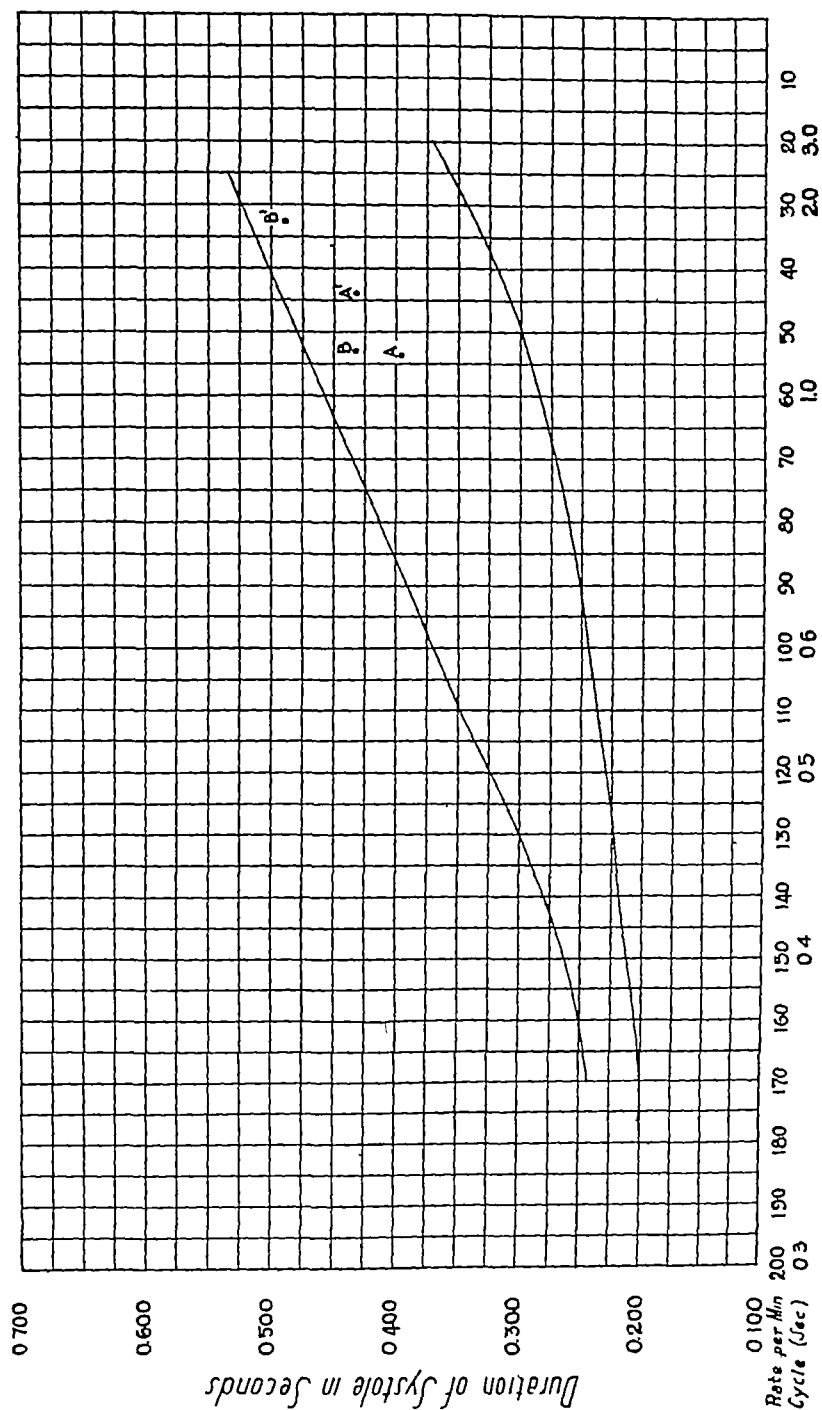
FIG 30 THE Q-T INTERVALS OF TWO CASES DURING ATRIOVENTRICULAR NODAL RHYTHM (A′, B′) AND DURING NORMAL RHYTHM (A, B)
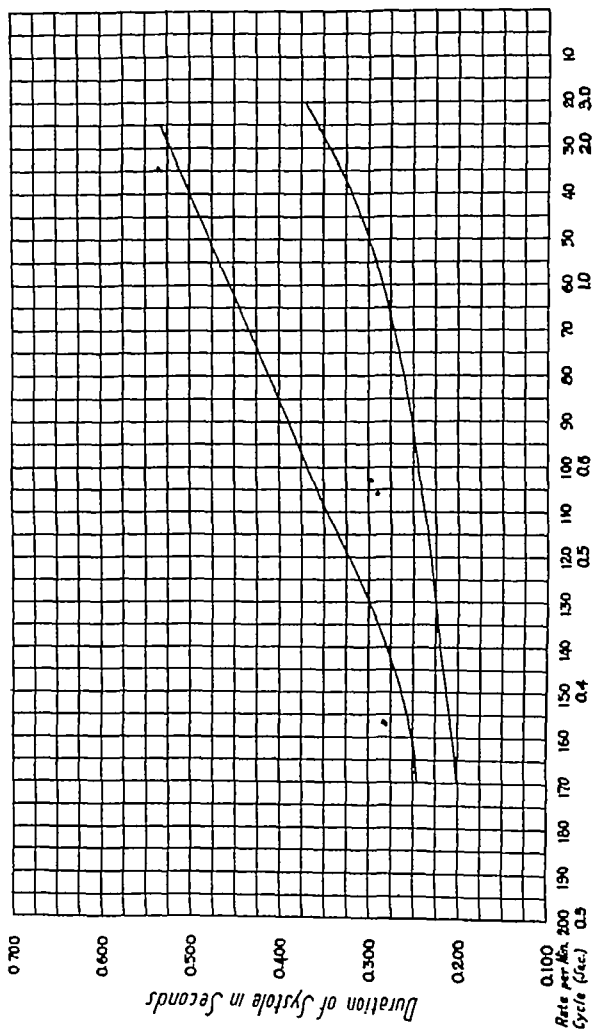
FIG 31 THE Q-T INTERVALS OF FIVE PATIENTS WITH LOW VOLTAGE, ONE OF WHOM HAD ALSO COMPLETE AURICULO-VENTRICULAR BLOCK

the normal upper limits   Of the three cases of complete auriculo-ventricular block without bundle branch block only one had a Q-T interval beyond normal limits and that was close to the line (fig 28)

K   Bundle branch block   Six further cases of bundle branch block (in addition to the three already noted under complete auriculoventricular block, and the one under aortic stenosis) have been studied Two showed right branch block and in one of these the Q-T interval was just below and in the other just above the outer limit of normal Three cases showed left bundle branch block, the Q-T interval in one of these was just within normal limits, one was on the border line itself, and one was very far beyond normal limits (nearly 0 7 second) In this last case, complete heart block was also present and so that instance should be added to the three already described with extremely long Q-T intervals under the heading of complete heart block   The sixth case showed a lesser grade of intraventricular block and its Q-T interval was barely within normal limits   However, all six cases were well above the normal average for duration of Q-T interval and near or beyond the upper normal limits   This finding is to be expected in view of the slow distribution of stimulus through the ventricles and inefficient onset of ventricular contraction (fig 29)

L   Atrioventricular nodal rhythm   Two cases of the rare atrioventricular nodal rhythm were studied, one also during normal rhythm and the other during auricular fibrillation   Both showed normal measurements for the Q-T intervals in each instance (fig 30)

M   Low voltage of electrocardiogram   Finally, five cases showing low voltage by electrocardiogram were studied   All showed Q-T intervals within normal limits except one with a very low pulse rate (38) due to complete heart block (fig 31)

SUMMARY AND CONCLUSIONS

1   A study is here reported of the measurements of the Q-T interval made by us on carefully selected electrocardiographic plates with the help of the Lucas comparator in 213 individuals, of whom 50 were normal to act as controls (20 men, 15 women and 15 children), and 163 were abnormal subjects to illustrate the effect of various pathological conditions

2, A brief review of the literature concerning the effect of various

factors on the duration of systole has been given, and references to papers listed in a bibliography

3 The interval from the beginning of the Q-R-S complex to the end of the T wave in satisfactory electrocardiograms may be considered as indicative of the duration of electrical systole, and perhaps is a more accurate measurement of actual systolic duration than any other The more accurately mechanical records of ventricular contraction are obtained, the more closely does the duration of mechanical systole approach that of electrical systole

4 The prime factor influencing the duration of both mechanical systole and the Q-T interval of the electrocardiogram has been found almost invariably by previous workers to be heart rate   This we have confirmed in our electrocardiographic study   The faster the heart rate the shorter the duration of systole and of the Q-T interval, although at faster heart rates the relative proportion of the heart cycle made up by systole steadily increases, as shown by the slope of the curve in figure 1   Whether the amount of blood entering the heart at various rates is a controlling factor is not clear

5 As a standard for judging the normal duration of electrical systole we have used a chart on which have been plotted according to heart rate and duration of the Q-T interval (coördinates) first the measurements of our 50 normal cases (fig 1) and then those of 190 normal cases from the literature (fig 2)   The outer limits of these 240 normal case measurements have then been joined by curve lines and the resulting figure has been used for judgment as to variation from the normal of our 163 abnormal cases   This method has seemed to us considerably more satisfactory than the application of any formula (no suitable formula has been devised)   Also our method has the advantage of rapid graphic illustration   Against the normal curves have been plotted the measurements of the Q-T interval in all of our 163 abnormal cases divided into groups according to the abnormal condition present

6 For the 240 normal cases the outer limits of normal for the Q-T interval varied from 0 480 to 0 300 second at a heart rate of 50 and heart cycle of 1 2 second to 0.262 to 0 214 second at a heart rate of 150 and heart cycle of 0 4 second   There is thus at slower pulse rates a wide normal variation perhaps due to variations in diastolic filling of the heart   The curve (fig 2) resembles somewhat a comet track

7 In normal individuals, the Q-T interval remained normal with change in position, exercise and digitalization

8 Of all the 163 abnormal cases, only two gave a duration of Q-T interval slightly shorter than normal and those were both cases with hyperthyroidism, one of whom also had auricular fibrillation and the other a well marked tachycardia   Both of these cases deviated but little from the normal (0 018 second in one and 0 025 second in the other)   No case in the entire series of 163 abnormal individuals gave a duration of systole much shorter than normal

9 A number of the 163 abnormal cases showed Q-T intervals longer than normal   Of these the most striking and uniform were four groups, first those cases with bundle branch block (six out of ten, the remaining four being on or close to the upper edge of normal), second those cases with very low blood serum calcium content (both of two cases), third the complexes of ventricular paroxysmal tachycardia (both of two cases) and of ventricular premature beats (all of four cases), and fourth four out of five cases of auricular paroxysmal tachycardia Bundle branch block, ventricular premature beats and ventricular paroxysmal tachycardia are alike in the abnormal and slow distribution of the excitation wave through the entire ventricular musculature The rates for the auricular paroxysmal tachycardia were so fast that we had no normal curves for comparison except by projection which is based on supposition only

10 The slow pulse of complete heart block (seven of nine cases, four of which seven, however, had bundle branch block also and one low voltage), of one case of sinoauricular bradycardia (out of seven), and of one case of partial auriculoventricular block (out of five) was associated with an abnormally long Q-T interval   The longest intervals of all were found in four cases of combined bundle branch block and complete heart block and they were 0 718 second (at rate of 36), 0 668 second (at rate of 34), 0 638 second (at rate of 23) and 0 632 second (at rate of 45)   These were the only Q-T intervals in the entire series of 213 cases which measured more than 0 6 second long

11 A few other scattered cases showed Q-T intervals slightly longer than normal   They were one case (out of five) of uremia, one case (out of nineteen) with a very large heart, one case (out of five) of

auricular premature beats (three others were on or near the upper normal limit line), and one case of auricular fibrillation with a very rapid ventricular rate Thus there were altogether of the 163 abnormal cases, 22 which showed overlong Q-T intervals and 18 of those showed either bundle branch block, very slow pulse rates, as in complete heart block, very fast pulse rates as in auricular or ventricular paroxysmal tachycardia, ventricular premature beat complexes, or too low a content of calcium in the blood serum

12 Of pathological conditions, cardiac enlargement, aortic regurgitation, aortic stenosis, mitral stenosis, congenital cardiac defects hypertension, diabetes mellitus, hyperthyroidism, hypothyroidism, acute infection, congestive failure, auricular fibrillation, atrioventricular nodal rhythm and low voltage in themselves did not affect the duration of the Q T interval in the series studied here

13 The measurement of the duration of the Q T interval of the electrocardiogram is apparently of little or no clinical value

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THE CALORIGENETIC ACTION OF THYROXIN AT DIFFERENT LEVELS OF BASAL METABOLISM IN MYXEDEMA

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INTRODUCTION

In the course of a study of the change in plasma volume accompanying treatment with desiccated thyroid in myxedema (1), it was noted that after the basal metabolic rate had been restored to normal, further increases in thyroid dosage up to four times the maintenance requirement produced proportionately much smaller increases in basal metabolism than did the maintenance dose. The effect at a normal level, in fact, appeared to be of about the same magnitude as that produced by giving thyroid to a normal individual

It seemed desirable to confirm these observations, using thyroxin intravenously instead of desiccated thyroid by mouth, in order to eliminate the possibility of non absorption of the latter from the gastro intestinal tract

METHOD

Accordingly, two patients with untreated typical myxedema were each given 10 mgm of thyroxin intravenously, and the influence on heat production and on clinical symptoms were compared with those following the same dose given to each patient at a later period when the basal metabolism was maintained at a normal level by desiccated thyroid (figs 1 to 6)

Both patients were kept in the hospital on routine diet throughout the "thyroxin curve" periods, with the exception of the second patient

who was not in the hospital during the time covered by the latter two-thirds of the first curve

It is very important to keep the patient for a long time on a given dose of desiccated thyroid before drawing the conclusion that a metabolic level is established on that dose The first patient was kept for four months on three grains of desiccated thyroid daily before the second injection of thyroxin   The second patient was kept for nearly two months on three grains, and then for four months on one and one-half grains of dessicated thyroid daily before the second injection of thyroxin, and on three grains daily for nine months before the third injection Moreover, after a level had been established on these doses at home, a change occurred after return to the hospital the metabolism determinations were usually lower, evidently due to the elimination of a rather long journey to the hospital before each test

In order to keep conditions constant throughout the experiment, *the daily doses of desiccated thyroid were continued following the second and third injections of thyroxin*

Basal metabolic rates were determined with the Roth-Benedict apparatus, using Aub-DuBois stndards

We used Squibb's thyroxin and Armour's desiccated thyroid

DATA

The course of the basal metabolic rate, of the pulse and of the weight in each instance, is depicted in figures 1 and 2   The effect of each injection on the basal metabolism is shown in detail in figures 3 and 4   The temperature reactions after each injection are shown in figures 5 and 6

The general clinical course of the toxic effects of an injection of thyroxin has been described by Boothby, Sandiford, Sandiford and Slosse (2), but detailed descriptions of the onset and duration of both toxic and beneficial effects in their relation to metabolism, have not been reported   For this reason, the clinical histories of these two patients are given in detail

Case 1 (figs 1, 3 and 5)   Lab No 4671   Mrs E G   Age 48   A case of untreated typical myxedema   On entering the hospital May 7th, 1927, her face was puffy, and she had swelling of the hands and legs which felt numb and clumsy She had gained 60 pounds in the last four years   Her tongue was thick and her
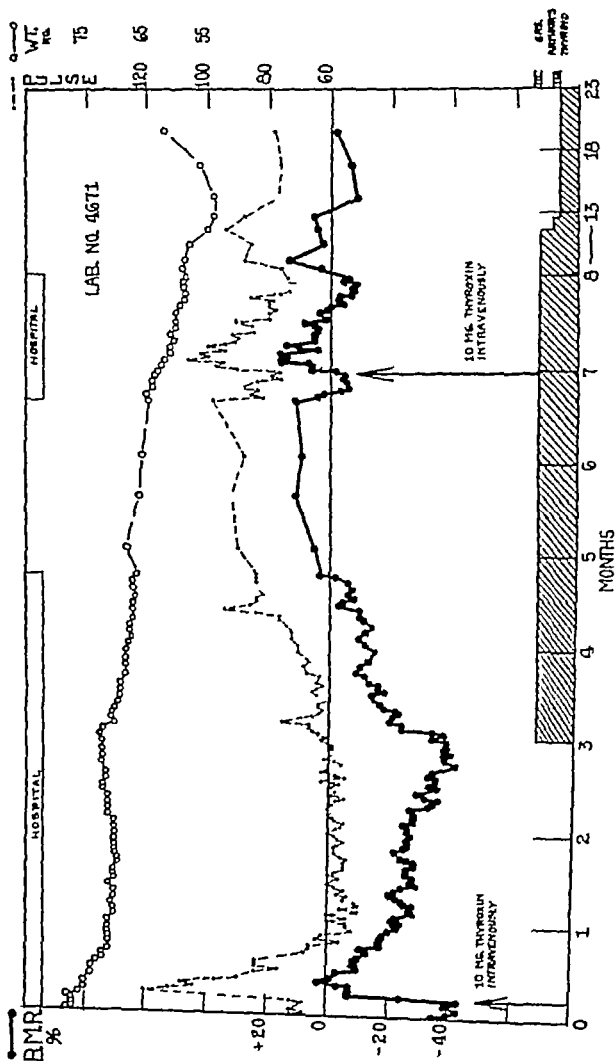
FIG 1 CASE 1   HEIGHT 170 cm   THE EFFECT ON BASAL METABOLIC RATE, PULSE AND WEIGHT OF THE INTRAVENOUS INJECTION OF 10 MGM OF THYROXIN, IN A PATIENT WITH MYXŒDEMA, AT DIFFERENT LEVELS OF BASAL METABOLISM

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Fig 2 Case 2 Height 160 cm Also Showing the Effect on Basal Metabolic Rate, Pulse and Weight of the Intravenous Injection of 10 mgm of Thyroxin, in a Patient with Myxedema, at Different Levels of Basal Metabolism (Minus 45 Per Cent, Minus 13 Per Cent and Minus 6 Per Cent)

Note that in both figure 1 and figure 2, the daily dose of desiccated thyroid which had been used to establish the higher levels of basal metabolism before the injections of thyroxin, was continued throughout the period that the thyroxin had any effect

hair very dry and brittle   Her skin was dry and scaly and showed marked p
mentation on the face and arms   Her voice was hoarse and her speech extrem
slow and deliberate   She felt sleepy, weak and cold all the time, and had so
dyspnea and palpitation on exertion   She never perspired   Her appetite v
poor and she was constipated   She was markedly slowed mentally, her mem
was very poor, and she was somewhat deaf   Her catamenia had been absent
six years   Her basal metabolic rate was minus 40 per cent

On May 14th at 3 p m she was given 10 milligrams of thyroxin intravenous
At 6 p m her skin felt warmer and her pulse had risen slightly   She had so
palpitation during the night.   Next morning she appeared brighter and tall
faster   The edema of her eyelids had noticeably diminished.   There was sli,
pain on moderate pressure of muscles of the right neck, upper arm and the lo
half of both legs   The following day she had marked palpitation and
as if she were burning up   She was nauseated and vomited three times
had a slight watery nasal discharge   On the 17th the edema of her eyel
was practically all gone   Her mouth and lips were parched   She was thir
and was passing more urine than usual.   There was no increase in mu
soreness   The next day she noticed that she felt less drowsy and that the s
on her face was softer, although it was still rough and dry on her hands   I
tongue felt as though it had 'more room" in her mouth.   On the 19th
muscle soreness had increased somewhat.   On the 20th it was noted that the s
of her hands was definitely smoother and softer   Her heart was much quie
Her facial expression had completely changed, due to the disappearance of ede
Her basal metabolism had reached its highest level of plus 3 per cent.   By
25th her skin was peeling all over her body   She felt brighter and stronger
moved about much more quickly   The muscle soreness was not as mark
The pigmentation of her skin was beginning to fade   Her hair was start
to fall out.   By the 30th the desquamation of her skin was very marked, par
ularly on her legs   Although still edematous at the ankles, her legs felt much
heavy and numb   She did not mind the cold and her appetite was improvi
Her voice was much less hoarse and she talked much more quickly than on ent
June 3rd the peeling of the skin was less marked and her hands did not feel as
Her ankles were still swollen slightly, but there was no edema of the face.
the 11th she stated that her hearing was better and that she could think m
quickly than before the injection of thyroxin   She could also move her fing
and toes more easily   June 15th she was still improving although her ba
metabolism had fallen to minus 28 per cent.   June 18th edema of the eyelids
gan to recur and she began to feel as if she would enjoy a nap after lunch
had had no ankle swelling for about a week   July 2nd she was still feeling v
and was able to walk around the hospital yard six times without resting—the f
time she had accomplished such a feat.   July 4th her voice was slightly hoarse
she talked a trifle more slowly   The pigmentation of her skin had faded still m
July 12th the skin had stopped peeling on her face but was still peeling marke

on her palms   She found it harder to keep balanced when walking   July 20th she felt more drowsy   July 29th her lower eyelids were markedly swollen on arising The thick heavy skin on the soles of her feet had begun to peel   Her basal metabolism was minus 33 per cent   August 8th she had some numbness of the fingers of her left hand   Her skin was slightly dry and it had just finished peeling on her hands   Her hair was not falling out   She was somewhat slowed up and her walking was a little unsteady   Her tongue was gradually becoming thick again Marked edema of the eyelids was present on arising, but there was no noticeable increase in edema elsewhere   She was not cold and said that she felt much stronger and brighter than when she entered the hospital   Her basal metabolism had reached its pre-injection level of minus 40 per cent

On August 9th she was started on 3 grams of Armour's desiccated thyroid daily Within two days she felt brighter and less sleepy   By August 16th the puffiness of her eyelids had diminished considerably and by August 29th it was gone   She felt tired however   By September 3rd the skin on her feet had stopped peeling She had slight muscle and joint pains   Her hair was falling out a little but new hair was growing in   October 1st she was discharged from the hospital with a normal basal metabolism   Her skin was smooth except for some peeling on her arms and hands   There was no edema of her eyelids   Her feet felt more supple and she was stronger and brighter than one month previously   The pigmentation on her arms and face had faded markedly   While home, she became more nervous, tired easily and appeared a little "washed out"   October 23rd she had her first normal catamenia for seven years   She was readmitted to the hospital November 29th   In a few days she felt more rested   Her level of basal metabolism was minus 4 per cent

She was given 10 mgm of thyroxin intravenously at 12 p m   December 8th Desiccated thyroid (3 grams daily) was continued   December 9th she noted no change except that she felt warmer, her heart pounded somewhat and the muscles of her left arm were slightly tender   That night she had a slight feeling of suffocation and had "catching pains" in her left axilla for about 15 minutes   December 10th she was a little nervous and "shaky"   By the 11th she was still feeling warmer and perspiring more   Her pulse became rapid only on exertion   She had anorexia and slight nausea but no vomiting   On the 12th she was very thirsty and was unsteady on her feet   December 13th, when in bed, she felt well, when walking about she got "shaky"   She had attacks of marked perspiration and her mouth felt dry and parched   December 14th she was tremulous, had intermittent muscle pains, and felt hot and fidgety   On the 15th she felt much better   Her appetite was improving   December 16th, although still somewhat fidgety, her muscle pains had disappeared   She noticed a marked increase in salivation since receiving thyroxin   She had practically no palpitation   On the 18th she still felt unusually warm.   The pigmentation of her skin had almost completely faded   December 20th she had no complaints   She felt better than she did on entry in that she was more rested   Her nervousness had practically disappeared   On the 29th she was clinically the same   Her skin was still

slightly more warm and moist than normal, although this had decreased noticeably within the last week. Her hair had begun to fall out about one week previously By January 1st, 1928 her basal metabolism had returned to its pre-injection level On the 7th she was discharged from the hospital, still taking 3 grains of desiccated thyroid daily After she went home, as she was tiring easily and was nervous and restless at night, perspired easily and had some precordial pain and palpita tion, her dose of thyroid was reduced to 2 grains daily on April 28th and to 1½ grains daily on May 25th On this smaller dose she gradually became stronger When last seen December 21st, 1928, she was well, had no palpitation, was not nervous, did not tire easily and weighed a little more than when taking 3 grains of thyroid daily There were no signs or symptoms of myxedema. Her basal metabolism was minus 2 per cent

Case 2 (figs. 2, 4 and 6) Lab No 4533 Mrs. M B Age 30 On entering the hospital February 19th, 1927, she was a classical case of untreated myxedema. She had been steadily gaining weight and had had no catamenia for five years Her tongue, face, hands, abdomen and legs were swollen Her hair was coarse and dry Her skin was very dry and scaling and she never perspired Her speech was slow She was always cold and drowsy Her appetite was poor, she was nauseated in the mornings and was very constipated. Her mental processes had become very slow and her memory poor She was ataxic and had frequent headaches The level of her basal metabolism was minus 45 per cent.

On March 3rd, at 3 p m. she was given 10 mgm of thyroxin intravenously At midnight she passed an unusually large amount of urine. All the next day she had a severe headache and marked nausea and anorexia, with increased thirst. She felt warm and objectively her face felt very hot There was a noticeable diminution in the edema of her eyelids She had slight pains all over her body and by evening her arm and leg muscles were sore on moderate pressure March 5th her headache was less severe. She felt very hot—her temperature was 104°F In the afternoon all her tissues all over her body were extremely sore She sweated profusely all night. Next day her muscle soreness was much less, although still marked in the leg muscles She was nervous and tremulous. The edema of her face had markedly diminished March 10th her basal metabolism had reached its highest level of minus 6 per cent For the first time in several days she was able to eat a moderate amount She talked faster and louder and felt much brighter She was still thirsty and passing an increased amount of urine. On the 12th the swelling had disappeared from her face and none was noticeable elsewhere in the body She had no headache She shook violently on trying to stand up She had had no nausea and vomiting for two days and her muscle soreness had subsided She looked much brighter The skin on her hands was more moist and smooth and the skin on her face and feet had just begun to peel March 14th she felt much improved and could walk better than for a week. Her appetite was fair By the 20th she was "feeling good," but she could not eat. On the 23rd her skin was still dry on her trunk, although definitely softer on her

on her palms   She found it harder to keep balanced when walking   July 20th she felt more drowsy   July 29th her lower eyelids were markedly swollen on arising   The thick heavy skin on the soles of her feet had begun to peel   Her basal metabolism was minus 33 per cent   August 8th she had some numbness of the fingers of her left hand   Her skin was slightly dry and it had just finished peeling on her hands   Her hair was not falling out   She was somewhat slowed up and her walking was a little unsteady   Her tongue was gradually becoming thick again   Marked edema of the eyelids was present on arising, but there was no noticeable increase in edema elsewhere   She was not cold and said that she felt much stronger and brighter than when she entered the hospital   Her basal metabolism had reached its pre-injection level of minus 40 per cent

On August 9th she was started on 3 grams of Armour's desiccated thyroid daily   Within two days she felt brighter and less sleepy   By August 16th the puffiness of her eyelids had diminished considerably and by August 29th it was gone   She felt tired however   By September 3rd the skin on her feet had stopped peeling   She had slight muscle and joint pains   Her hair was falling out a little but new hair was growing in   October 1st she was discharged from the hospital with a normal basal metabolism   Her skin was smooth except for some peeling on her arms and hands   There was no edema of her eyelids   Her feet felt more supple and she was stronger and brighter than one month previously   The pigmentation on her arms and face had faded markedly   While home, she became more nervous, tired easily and appeared a little "washed out "   October 23rd she had her first normal catamenia for seven years   She was readmitted to the hospital November 29th   In a few days she felt more rested   Her level of basal metabolism was minus 4 per cent

She was given 10 mgm of thyroxin intravenously at 12 p.m December 8th   Desiccated thyroid (3 grams daily) was continued   December 9th she noted no change except that she felt warmer, her heart pounded somewhat and the muscles of her left arm were slightly tender   That night she had a slight feeling of suffocation and had "catching pains" in her left axilla for about 15 minutes   December 10th she was a little nervous and "shaky "   By the 11th she was still feeling warmer and perspiring more   Her pulse became rapid only on exertion   She had anorexia and slight nausea but no vomiting   On the 12th she was very thirsty and was unsteady on her feet   December 13th, when in bed, she felt well, when walking about she got "shaky "   She had attacks of marked perspiration and her mouth felt dry and parched   December 14th she was tremulous, had intermittent muscle pains, and felt hot and fidgety   On the 15th she felt much better   Her appetite was improving   December 16th, although still somewhat fidgety, her muscle pains had disappeared   She noticed a marked increase in salivation since receiving thyroxin   She had practically no palpitation   On the 18th she still felt unusually warm.   The pigmentation of her skin had almost completely faded   December 20th she had no complaints   She felt better than she did on entry in that she was more rested   Her nervousness had practically disappeared   On the 29th she was clinically the same   Her skin was still

knee jerks, was probably unrelated to her myxedema   There was no essential change in her basal metabolism.

November 28th, she was given 10 mgm of thyroxin intravenously at 8 p m. Desiccated thyroid, grains 1½ daily, was continued.   During the night her head began to ache   Next morning her headache was gone, but she felt more nervous and restless   In the evening she vomited.   On the 30th she felt exceedingly warm   Her skin was hot although not moist, and her temperature was 101.5°F She had a headache and was thirsty   She was not passing more urine   Decem ber 1st she had no headache, nausea or fever, but she felt weaker and more nervous On the 2nd her nervousness had decreased.   On the 3rd she felt "fine ' and con tinued thus, except for a transient attack of aches and pains on December 15th Her basal metabolism reached its pre injection level of minus 13 per cent about December 21st.   On the 31st she was discharged from the hospital, with a basal metabolism of minus 18 per cent   She felt much brighter and livelier than on admission and had no ataxia.   One and one-half grains of thyroid was continued at home   On this dose she felt well until February 1928, when she had begun to be dopey and drowsy again   Thyroid was increased to 3 grains daily   On this dose her metabolism rose to normal, she became more nervous and tired more easily Her appetite was poor   She had no palpitation   She reentered the hospital November 17th.   There were no signs or symptoms of myxedema and her basal metabolic level was minus 6 per cent.

She was given 10 mgm. of thyroxin intravenously at 6 p.m., November 26th. At about 10 o'clock that night a dull headache which she had had for several days, became much worse, and she became nauseated.   Next morning there was some tenderness of the leg and arm muscles this became more pronounced towards evening   She was very nervous, had a tremor, was unusually thirsty and per spired a great deal.   That night she had severe pain about the umbilicus and vomited four times   On the 28th her nausea and headache were less marked, and her skin was not so warm.   Her temperature was 100°F   Her muscles were still sore.   On the 29th she was much less nervous and she had no tremor or nausea.   Muscle tenderness was much less marked.   Her skin temperature was about normal   November 30th she had some palpitation and nausea in the morn ing, and she felt nervous and jumpy   In the afternoon her heart was quieter Her skin felt warm and her leg muscles were still quite tender   She was drinking more water and passing more urine   December 1st, her appetite, which had been poor since injection, improved.   On the 2nd, muscle tenderness was confined to the legs, which were moderately tender   She had no cardiac symptoms   De cember 5th she was feeling well.   The muscles of her right calf were still slightly tender   By the 7th muscle tenderness had completely disappeared.   From then on, her progress was uneventful, except for a mild upper respiratory infection about December 17th.   Her basal metabolism reached its pre injection level of minus 6 per cent about December 19th   She was discharged on 3 grains of thyroid daily, on the 21st.   March 11th, 1929, when last seen, she was well.

There were no signs or symptoms of myxedema and her basal metabolism was plus 2 per cent
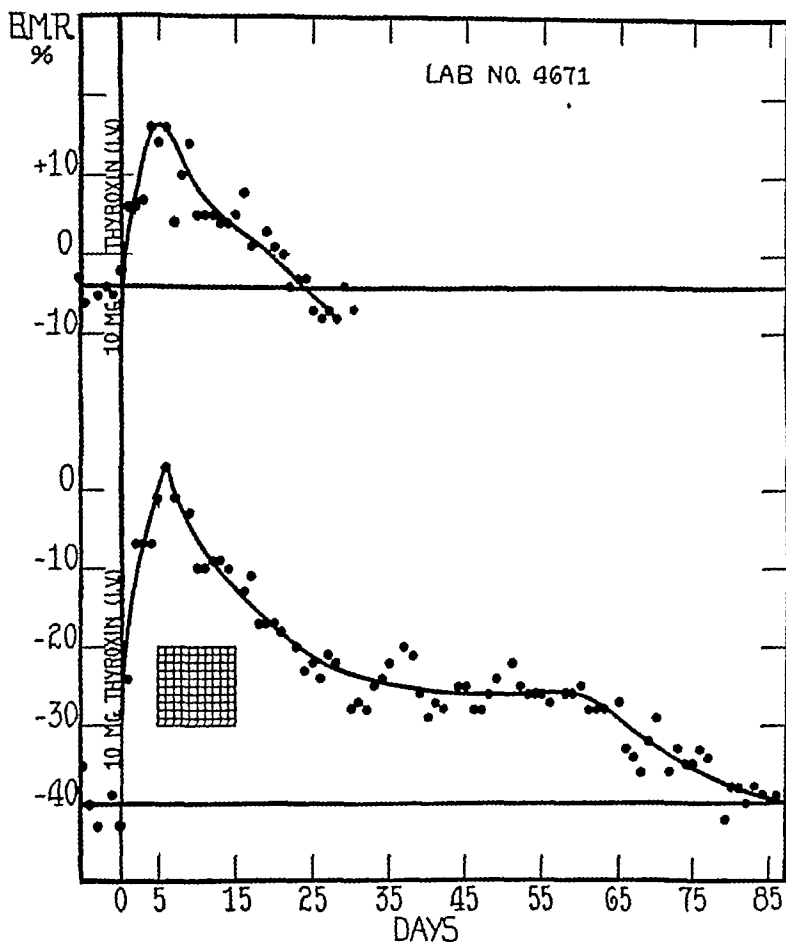
FIG 3 CASE 1   SHOWING IN DETAIL THE MARKED DIFFERENCES IN THE MAGNI-
TUDE AND DURATION OF THE INCREASE IN BASAL METABOLISM FOLLOWING THE
INTRAVENOUS INJECTION OF 10 MGM OF THYROXIN, AT DIFFERENT METABOLIC
LEVELS (MINUS 40 PER CENT AND MINUS 4 PER CENT)

*Comparison of effect on basal metabolism of the intravenous injection of
10 mgm of thyroxin when the patients were myxedematous
and when they were normal*

It may be noted from figures 3 and 4 that, when the patients were myxedematous, the increase in basal metabolism following the intra-
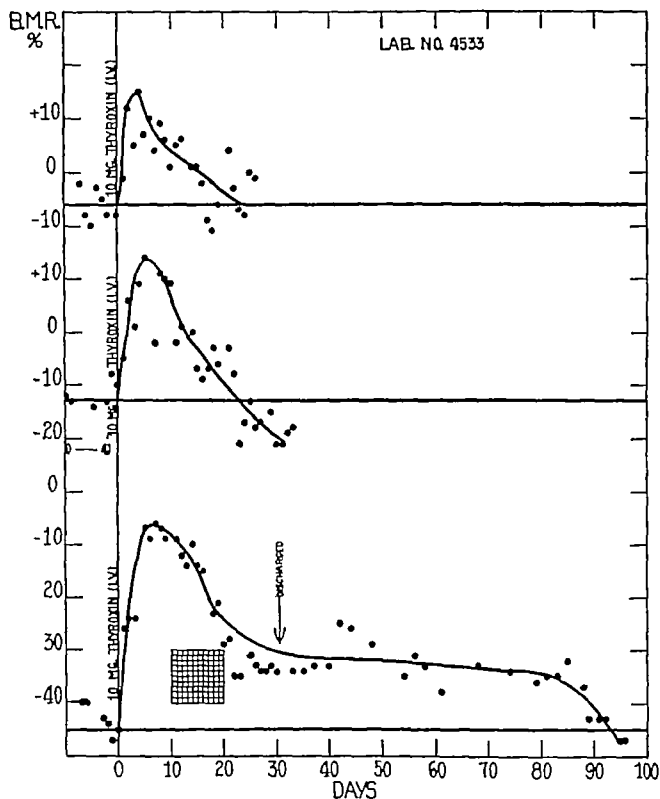
Fig 4 Case 2   Also Showing in Detail the Marked Differences in the Magnitude and Duration of the Increase in Basal Metabolism Following the Intravenous Injection of 10 mgm of Thyroxin, at Different Metabolic Levels (Minus 45 Per Cent, Minus 13 Per Cent and Minus 6 Per Cent)

venous injection of 10 mgm of thyroxin was of much greater magnitude and of much longer duration than when the patients were normal These differences are summarized in table 1

The total excess heat produced by each injection of 10 milligrams of thyroxin was estimated as follows

The daily metabolic rates were plotted on graph paper ruled in small squares (sample insert in each figure), each small square representing a change of 1 per cent in the standard normal basal calories

TABLE 1

Summary of effect on basal metabolism produced by the intravenous injection of 10 mgm of thyroxin at different metabolic levels in myxedema

Case number	B M.R. before injection	Surface area before injection	Number of squares	Total excess calories	Excess calories per square meter body surface	Per cent rise in B M R. per milligram injected	Per cent rise in B M.R. per square meter body surface per milligram injected	Time required for B M.R. to rise to maximum	Total length of time B M.R. was affected	Time required for B M.R. to drop from maximum to pre-injection level
	per cent	*sq m*						*days*	*days*	*days*
1 {	−40	1 89	1,375	22,455	11,880	4 3	2 3	6	86	80
	−4	1 74	221	3,320	1,910	2 0	1 2	4	24	18
2 {	−45	1 65	1,458	21,075	12,770	3 9	2 4	5	93	85
	−13	1 57	303	4,165	2,655	2 7	1 7	5	23	18
	−6	1 60	201	2,815	1,760	2 1	1 3	4	23	19

per square meter per 24 hours   A curve was drawn through the points thus plotted, to denote the actual course of the metabolism The base line was the level of the metabolism before the injection of thyroxin   The number of squares contained in the area bounded by the base line and the curve was counted   The difference in the number of squares in the graphs at different metabolic levels gives a fairly close estimate of the difference in the magnitude of the response at each level   but to bring each response to terms of total excess heat production, the normal basal metabolic rate (Aub-DuBois standards) in calories for 24 hours was multiplied by the number of squares and the result divided by one hundred

Example
Case 1
Basal metabolic rate before injection, minus 40 per cent
Standard normal calories per square meter per hour = 36 0
Body surface before injection = 1 89 square meters
If metabolic rate were standard normal before injection, total basal calories for
24 hours would be 36 0 × 24 × 1 89
  Number of squares in graph = 1375

Total excess calories produced by 10 mgm of thyroxin $= \dfrac{36\ 0 \times 24 \times 1\ 89 \times 1375}{100}$

= 22,455
  Total excess calories per square meter produced by 10 mgm. of thyroxin =
$\dfrac{36\ 0 \times 24 \times 1375}{100} = 11{,}880$

The method used is rough, but is sufficiently accurate, considering
the number of other variables which enter into the situation, to show
the marked differences in reaction which occur at different levels of
basal metabolism.[3]

When the patients were myxedematous with basal metabolic rates
of minus 40 and minus 45 per cent, the total excess heat produced
by the injection of 10 mgm of thyroxin was $\dfrac{22{,}455}{3{,}320} = 6\ 8$ times and

$\dfrac{21{,}075}{2{,}815} = 7\ 5$ times as great respectively as when they were normal

with rates of minus 4 and minus 6 per cent

It is of interest that the ascending portions of the curves
occupied about the same length of time (4 to 6 days) at both the
normal and the myxedematous levels, being possibly a little shorter
at the normal level

The rate of increase in heat production was greatest immediately
following injection, particularly at the myxedematous level. Thus
in case 1 it had risen 16 out of 43 points within 18 hours after injection
and 33 points within 42 hours   it then remained at a level for another
48 hours before starting to rise to its maximum, which was reached
138 hours after injection   In case 2 it had risen 20 out of 39 points

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[3] In each calculation the figure used for surface area is that of the patient just
before injection   The ensuing changes in area are not taken into account, because
the influence on final results is so slight.

within 18 hours after injection, and then remained at about the same level for another 48 hours before starting to rise to its maximum, which was reached 114 hours after injection

The descending portions of the curves occupied a much longer time when the injection was given at the myxedematous levels (80 to 85 days) than when it was given at the normal levels (18 to 19 days) At the normal levels, the initial part of the fall was perhaps a little steeper than the last part   The long plateau, at a level a little above the base line, which was an outstanding feature of the descending portion of the curve when the injection was given at a myxedematous level, was missing

Comparison of effect on clinical condition of the intravenous injection of 10 mgm of thyroxin when the patients were myxedematous and when they were normal

As the clinical histories show, there were two outstanding differences

1 The symptoms attributable to intoxication, viz , vomiting, anorexia, fever, headache, palpitation, increased thirst, tremor and muscle tenderness, were all more marked when the patients were myxedematous than when they were normal

2 The clinical improvement at the myxedematous level was striking at the normal level, the only effect was a toxic one

It is of interest that the degree of muscle tenderness and the increase in temperature following injection appear to vary directly with one another   Thus, in the first patient, there was only slight muscle tenderness even at the myxedematous level, and the temperature was only slightly elevated (fig 5) at the myxedematous and not at all at the normal level   In the second patient, however, the muscle tenderness was very severe at the myxedematous level and the temperature was correspondingly high, viz , 104 degrees Fahrenheit (fig 6) at the normal level both were less affected, but nevertheless were as marked as in the first patient at the myxedematous level   These observations suggest that the muscle tenderness and increase in body temperature following the injection of thyroxin, may be due to the same cause, i e , destruction of tissue

A striking feature was the rapidity with which the clinical reaction

appeared following the injections   This was shown particularly when
the injection was given at the myxedematous level   Thus, in case 1,
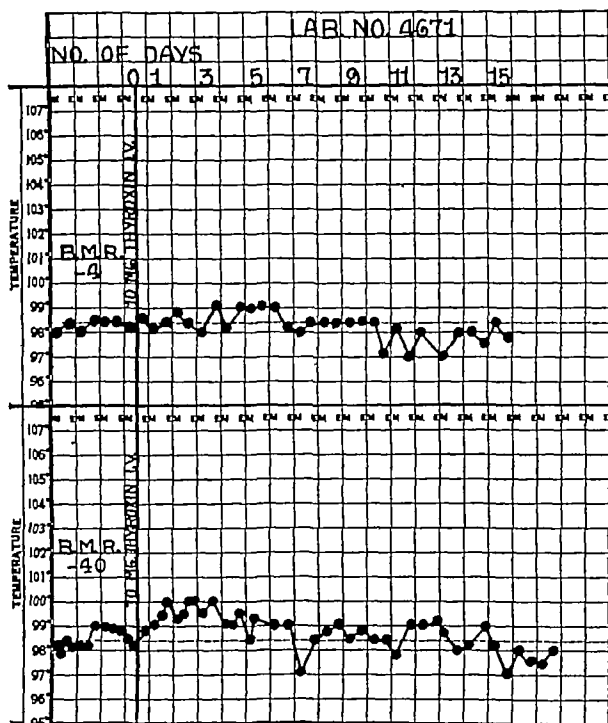
FIG 5 CASE 1   THE EFFECT ON BODY TEMPERATURE OF THE INTRAVENOUS
INJECTION OF 10 MGM. OF THYROXIN AT DIFFERENT METABOLIC LEVELS (MINUS
40 PER CENT AND MINUS 4 PER CENT)

as evidenced by increased pulse rate and temperature, the reaction
had begun 3 hours after injection   In case 2, where the patient was
not followed closely immediately afterward, all that was noted was
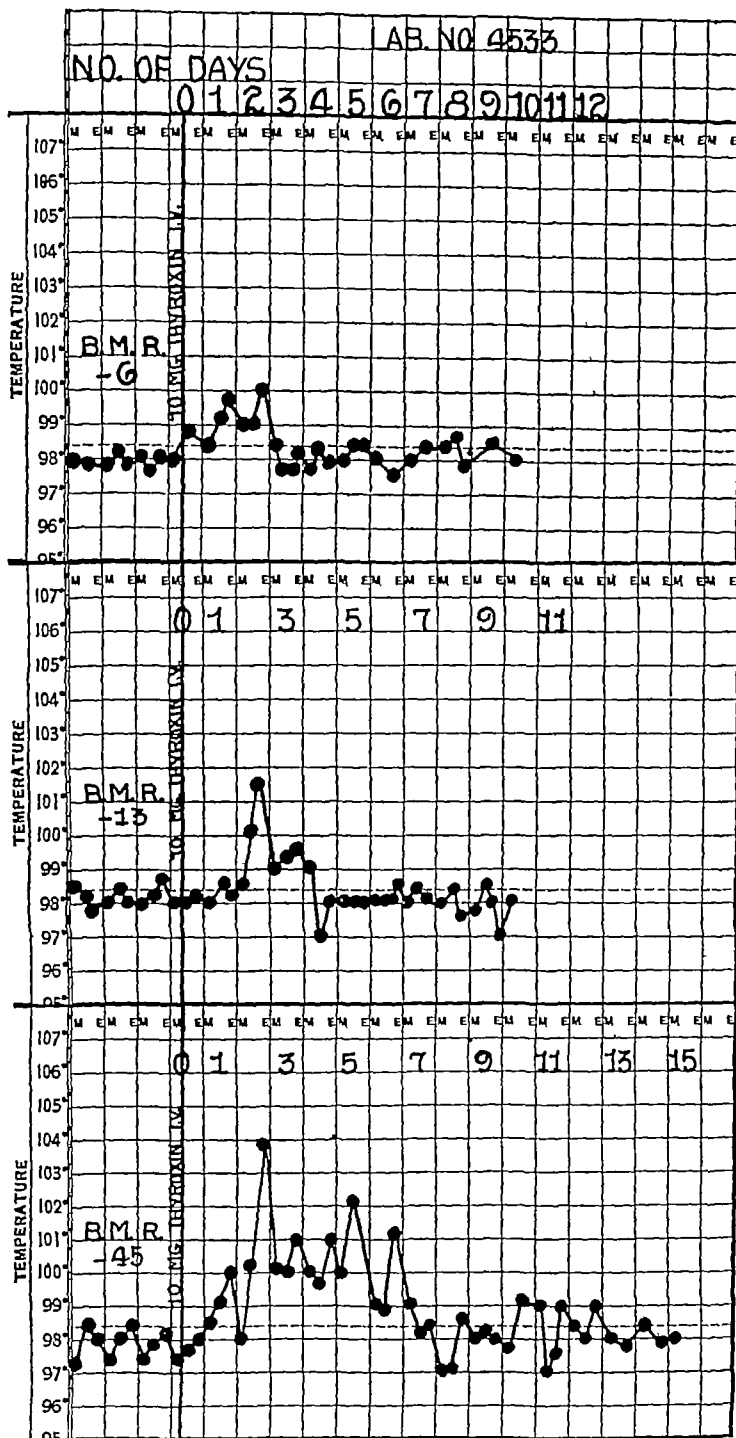
FIG 6 CASE 2 ALSO SHOWING THE EFFECT ON BODY TEMPERATURE OF THE INTRAVENOUS INJECTION OF 10 MGM. OF THYROXIN AT DIFFERENT METABOLIC LEVELS (MINUS 45 PER CENT, MINUS 13 PER CENT AND MINUS 6 PER CENT)

TABLE 2

Comparison of clinical condition of patients before the injection of 10 mgm of thyroxin and after its effect on basal metabolism had worn off (myxedematous level)

	Case 1		Case 2	
	Before injection of 10 mgm. thyroxin. B.M.R. —40 per cent	86 days after injection of 10 mgm. thyroxin. B.M.R. —40 per cent	Before injection of 10 mgm. thyroxin. B.M.R. —45 per cent	93 days after injection of 10 mgm thyroxin. B.M.R. —45 per cent
Weight	78 kgm	72 kgm	63 kgm.	58 Lgm
Pulse	68	62	62	54
Edema	Face, legs hands and abdomen swollen	Eyelids swollen No noticeable edema elsewhere Skin on hands loose	Face, legs, hands and abdomen swollen	Eyelids slightly swollen. No edema of legs or hands. Abdomen slightly swollen
Skin	Very dry and scaly Marked pigmentation of face and arms	Moderately dry Pigmentation markedly faded	Very dry and scaly	Slightly dry
Hair	Very dry and brittle	Not dry	Very dry and coarse	Not dry
Tongue	Large and thick	Slightly thick	Large and thick	Normal
Voice	Hoarse	Slightly hoarse	Hoarse	Slightly hoarse
Gait	Markedly unsteady	Slightly unsteady	Markedly ataxic	Spells of ataxia
Slowness				
Of movement	++++	+	+++	+
Of speech	+++	+	++	+
Of thought	++	+	+++	+
Hearing	Poor	Good	— —	— —
Memory	Very poor	Fair	Very poor	Fair
Sensitivity to cold	+++	0	++++	++
Numbness of extremities	++	+	— —	— —
Drowsiness	++++	++	++++	++
Weakness	+++	+	+++	+
Headaches	0	0	+	+
Appetite	Poor	Fair	Poor	Rather poor
Nausea	0	0	+	+
Constipation	+++	+	+++	++

polyuria 9 hours after injection The day after injection there was a noticeable diminution in the edema of the eyelids in both instances the patients looked brighter and moved a little more quickly The second patient, after the second injection (basal metabolic rate before injection minus 13 per cent) developed a headache in ·about 4 to 6 hours, and after the third injection (basal metabolic rate before injection minus 6 per cent), a headache which had been present for several days, became worse 4 hours later and she became nauseated

The height of the intoxication was reached on the second day following all injections except the second one in the first patient In this instance, it was reached on the fourth day after injection In both patients at the myxedematous level, all the symptoms of intoxication had begun to abate after the fifth day Following the second injection in the first patient, the peak of the reaction was over in six days and following the last two injections into the second patient, the symptoms attributable to intoxication had practically disappeared by the fifth day

When the patients were myxedematous, the clinical benefit of the injection of 10 mgm of thyroxin was not at its height until three weeks after injection, and at this time the weight was still falling although the basal metabolism had dropped to minus 20 per cent in case 1 and minus 28 per cent in case 2 In the first patient, whose hospital routine was not disturbed, this benefit continued unabated for three more weeks, by which time she had lost a little more weight and her basal metabolism had fallen to minus 28 per cent In both patients the reversion to myxedema was very gradual and by no means complete When their basal metabolic rates had returned to their pre-injection levels of minus 40 and minus 45 per cent respectively, both patients showed very few manifestations of thyroid deficiency and moreover weighed 6 and 5 kilograms less respectively than before injection, although at these same levels of metabolism they had previously been classical cases of myxedema Table 2 illustrates this point.

<div align="center">DISCUSSION</div>

<div align="center">*A Differences in excess heat production*</div>

That the decrease in the magnitude of the reaction to the intravenous injection of 10 mgm of thyroxin at a normal level of basal

metabolism as compared with a myxedematous level of metabolism, is not due to the establishment of a tolerance to thyroid substance, is upheld by the following considerations

1 We have never encountered a case of myxedema, in which a dose of desiccated thyroid which was definitely established by administration for a long enough period of time to be sufficient for maintenance, had to be increased later because of the development of a tolerance to the substance.

2 The response of normal and obese individuals to desiccated thyroid by mouth (1) (3) (4) (5) or to thyroxin given intravenously (2) (6) is less than that of patients with myxedema

A more reasonable explanation of the decrease observed, is that at the myxedematous level, the thyroxin is supplying a marked, perhaps complete deficiency of thyroid gland secretion, whereas at a normal level, thyroxin is being given in excess   The body would be expected to deal with these different situations in a different manner, in the latter instance bringing into play some mechanism which would tend to keep conditions constant

By injecting the thyroxin intravenously, it can be made certain that it is completely absorbed by the blood stream but the amount, time and method of its absorption by other tissues and its excretion from the body, under different circumstances, are practically unknown Kendall (7) states that within fifty hours after 200 milligrams were injected into the saphenous vein of a dog, 43 per cent of the total iodine contained in the thyroxin was excreted in the bile and 13 per cent in the urine. In this case the thyroxin was given in great excess so that these figures probably do not represent the per cent of a 10 mgm dose that is excreted unused when given to a patient with myxedema.

It is reasonable to assume that under normal conditions, when the rate of cell metabolism is more rapid than in the myxedematous condition, the excretion of thyroxin may also be more rapid   This may be at least one element in the mechanism which, at a normal level of metabolism, guards against the effects of excess dosage of thyroxin

Incidentally, a curve denoting the effect on basal metabolism of an intravenous dose of thyroxin represents only the effect of whatever fraction of that dose is not eliminated from the body unused at one

time or another during the course of the observations   One would expect this fraction to vary under different circumstances

Plummer and Boothby (8) state that in myxedema, 1 milligram of thyroxin injected intravenously raised the basal metabolism an average of 2 8 per cent   Their table shows that they divided their myxedematous patients into groups according to the metabolism, which ranged from below minus 35 per cent to above minus 14 per cent The lower the metabolism, the larger the dose of thyroxin administered Their results ranged irregularly from 1 6 to 3 5 per cent increase in metabolism per milligram of thyroxin injected   In the groups with metabolic rates "above minus 14 per cent," "minus 15 to minus 19 per cent" and "minus 35 per cent and below" (the only ones in which significantly different doses were given at the same metabolic level), the results suggest that the greater the dose the less the increase in metabolism per milligram of thyroxin injected   That the size of the dose may affect such results is indicated by Kendall's (9) observation that the injection of one enormous dose (200 mgm) into a dog had no more clinical effect than a single small dose   While 2 8 per cent rise per milligram is the proper *average* for Plummer and Boothby's observations throughout their metabolic range, and may be for such observations in general, it should be understood that the variations allowed for by this figure are within rather wide limits   Our findings indicate that for a 10 mgm dose, the variation may range from a rise of 4 3 per cent per milligram to a rise of only 2 per cent per milligram, depending upon the initial level of the basal metabolism within the range of minus 45 to minus 4 per cent

Boothby, Sandiford, Sandiford and Baldes (10) have attempted to reduce the relation between the size of the dose and the excess heat production to mathematical terms   On the basis of their interesting data most of which are charted in another article (2), and which were made on one myxedematous patient, they tentatively estimate that the continued administration of an average intravenous dose of 0 25 mgm of thyroxin daily raised the heat production approximately 525 calories above the myxedematous basal level of 1100 calories, an average dose of 1 mgm daily raised it approximately 825 calories and 2 mgm daily, approximately 1020 calories   As this relation between

dose and extra heat is a straight line on logarithmic paper, they suggest the following equation to represent it

$$\text{Log } H - n \log T = K$$

where H represents the excess calories and T the daily dose of thyroxin necessary to maintain H

As the authors evidently realize however, judging by the fact that they offer it as only a preliminary suggestion, there are possibilities for error in this generalization   The most serious criticism of their formula is that the actual doses were not 0 25, 1 and 2 mgm daily, but averages of different doses given at longer intervals, viz , 1 to 14 days   The results of such a program of medication may differ from the results that would be obtained from actually giving 0 25, 1 and 2 mgm daily, on account of possible differences in the rate of excretion of thyroxin   Moreover, the per cent of thyroxin excreted unused when the dose is 2 mgm daily may be different from that excreted unused when the dose is smaller   Another consideration is that there may be a different relationship between excess heat and dose at levels of basal metabolism below standard normal, from that which obtains above normal

B Differences in length of descending portions of the curves

Plummer (6) makes the general statement that, in a thyroidless individual, the effect on basal metabolism of an intravenous injection of thyroxin—sufficient to bring the metabolism to normal—wears off in 5 to 7 weeks   In another article (11), he states that the effect of 14 mgm. may not have completely worn off in 8 weeks   Boothby, Sandiford, Sandiford and Slosse (2) report two curves on a myxedematous patient   The first represents the rate of decrease of the metabolism which had been raised by 3 9 grams of desiccated thyroid by mouth, and occupies a minimum of 45 days   The other represents the rate of disappearance of the effect on metabolism of an average intravenous dose of 1 mgm of thyroxin daily for about a month, and occupies a theoretical minimum of about 55 days   The curve is not plotted on actual data in the last half of its course. In another article (12) Boothby and Sandiford state that the time required for the heat production to decrease from the maximum to one-

tenth of this amount, is found by extrapolation to be usually between 30 and 70 days for thyroxin W A Plummer (13) states that the effect of an intravenous injection of thyroxin on metabolism in myxedema sometimes lasts for 10 weeks Baumann and Hunt (14) on the basis of their experiments on "the specific dynamic action of glucose" following complete thyroidectomy in rabbits, concluded that it took about 65 days for the thyroid secretion in the tissues to become exhausted Magnus Levy (15) found in his myxedematous patient that it took about 80 days for the effect of thyroid feeding on basal metabolism to wear off

As may be seen from figures 3 and 4, the level of the metabolism at the time of injection of thyroxin has a very important bearing on the time occupied by the decrease in basal metabolism, and consequently upon the total amount of excess heat produced This may explain some of the differences noted by various observers in the duration of the descending portion of the curve

C Possible significance of the lag of clinical effects behind basal metabolism after injection of thyroxin when the patients were myxedematous

The marked lag in disappearance and reappearance of clinical signs and symptoms of myxedema behind the rise and fall in basal metabolism illustrates the striking slowness with which the chemical changes in tissues that are characteristic of myxedema occur In terms of basal metabolic rate, the effect of the injection of 10 mgm of thyroxin in myxedema is completed in 90 days, but in terms of general well-being, the effect lasts for some time longer—probably several months If the latter effect be due to some residual thyroxin in the tissues, then the rate of destruction of thyroxin has never been even approximately determined If it is merely an expression of some mechanism which thyroxin has set into play and which is acting long after the thyroxin itself has become exhausted, then there is no guarantee that at least part or possibly all of the descending portion of the basal metabolism curve is not of the same nature Possibilities such as these make one hesitate to apply formulae to the descending parts of the curves of metabolism and

to use the results to calculate the amount of thyroxin in the body [4] Even if there were sufficient data, well controlled as to dosage and level of metabolism at the time of injection, one would be at a loss to know at what point all the thyroxin actually disappeared from the reaction

Boothby, Sandiford, Sandiford and Baldes (10) state that the data of the descending portions of their three curves on one myxedematous patient lie within the experimental error of an exponential curve of the following formula

$$H + H' = 1660 \times 10^{-0.024t} + 1150$$

where H = excess heat calories due to thyroxin and 1660 is an arbitrary value for zero day, $10^{-0.024}$ is the exponential constant, t the number of days following the zero day and H' is 1150, the heat production of the myxedematous subject before the administration of thyroxin The excess heat production when plotted on semi-logarithmic paper against time fell on a straight line and could be expressed by the above formula. Therefore, they tentatively assumed that this excess heat was dependent on some function of the concentration of thyroxin in the body They further state that "if we assume that the excess heat is directly proportional to the concentration of thyroxin in the body then the disappearance of thyroxin from the body obeys the same law as the excess heat production and the rôle of the thyroxin is identical with that of a specific catalyst and follows the law of monomolecular reactions "

Rabinowitch (16) points out that while the descending portion of the curve possibly may be explained on such a basis, the ascending portion cannot be. "Since we are dealing with a reaction which increases in magnitude for a number of days after the administration of a single dose of the drug, it is obvious that the concentration of that

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[4] Inasmuch as the rate at which thyroxin is used and the magnitude of its effect appear to depend upon the level of the basal metabolism before injection and the size of the dose given, inasmuch as the basal metabolism does not necessarily record the concentration of thyroxin in the tissues, and inasmuch as the rate and time at which it disappears from the reaction when its administration is stopped cannot be determined at present, we find it difficult to understand how Plummer (11) calculated that there are about 14 mgm. of thyroxin in the body of a normal man His method of making this calculation is not recorded.

drug in the tissues is not the only governing factor" He suggests that we are dealing with a form of growth in which the rate of increment is at any particular instant proportional to the magnitude of that which is increasing   Thus the successive rates of heat production at the end of a series of intervals would form the terms of a geometrical progression, according to the formula

$$Q_t = Q_o \, e^{kt} \text{ or } k = \frac{2\,302}{t} \log_{10} \frac{Q_o + X_t}{Q_o}$$

where Q_o = the original quantity, k = constant, Q_t = the amount to which Q_o has grown in time t and X_t = the increment during time t "If the law is applicable, it should be possible to predict the heat production at the end of any period of observation, once the constant of increment is known "   When a constant value for k was used throughout the period of increment, the calculated results agreed reasonably well with the observed results   If values for k were calculated at different intervals, however, a marked inconstancy was noted   He found a similar variation in the value for k in the descending portion of one of the curves of Boothby and Rowntree (17)   He therefore concluded that "we are either not dealing with a reaction that follows the unimolecular law, or, as in the case of ferments, the fall in k may be attributed to destruction of the catalyst   If, however, the latter explanation is accepted, it is difficult to reconcile the increase in the rate of heat production which follows with the view that the heat production is a direct function of the concentration of thyroxin in the tissues "   He suggests the possibility that, during the reaction, certain compounds are formed which are catalytic and may account for the prolonged effect following the administration of thyroxin

The slope of the descending portions of our curves would appear to agree with the contention of Rabinowitch that the reaction is not a monomolecular one and we are of the opinion that it is at present hopeless to give a satisfactory theoretical and mathematical treatment of so complex a biological phenomenon

D Bearing of our results on the treatment of myxedema

We have frequently noticed that after the basal metabolism has been raised to a normal level in patients with myxedema, it is often possible,

by increasing slightly the oral dose of thyroid ($\frac{1}{2}$ to $1\frac{1}{2}$ grains of Armour's thyroid daily), to produce unpleasant clinical symptoms without much increase in basal metabolism   For instance, in our first patient, 3 grains of desiccated thyroid were continued daily for nearly five months after the second injection of thyroxin   As shown in figure 1, after the effects of the injection had worn off, although the basal metabolism remained approximately normal (plus 2 to plus 4 per cent, with the exception of one determination of plus 13 per cent shortly after she went home), her weight dropped steadily   She developed some palpitation and precordial pain, was nervous, fatigued easily, perspired more than usual and looked worn out   On reducing the dose of desiccated thyroid to $1\frac{1}{2}$ grains daily, her metabolism was still within normal limits (minus 9 to minus 2 per cent), and she gained weight   Her symptoms of thyroid intoxication disappeared and there was no evidence of myxedema.

Our results with thyroxin are in harmony with this observation   The frequent lack of effect on metabolism of a slight excess of desiccated thyroid when given at a normal level of metabolism is similar to the very moderate effect produced at this level by a large excess (10 mgm) of thyroxin

Inasmuch as a small excess of thyroid is not necessarily reflected in the level of the basal metabolism, the latter is not the only factor to be considered in gauging the proper maintenance dose for a patient with myxedema.   The determination of the dose of desiccated thyroid must be in part based upon a careful clinical study of the patient

SUMMARY

In two patients with myxedema, the intravenous injection of 10 mgm of thyroxin produced more marked effects at a low level of metabolism (minus 40 and minus 45 per cent) than at a normal level of metabolism (minus 4 and minus 6 per cent respectively)   This is shown by the following facts

1 The total excess heat produced was about seven times as great viz, 22,455 calories vs 3,320 calories in the first case, and 21,075 vs. 2,815 calories in the second case

2 The per cent rise in basal metabolism per milligram of thyroxin

injected was about twice as much, viz , 4 per cent per milligram vs 2 per cent per milligram

3   The length of time occupied by the descending portion of the metabolism curve was about four times greater, viz , 80 to 85 days vs 18 to 19 days

4   The unpleasant clinical effects, including elevation of temperature, were more marked

5   The beneficial clinical effects were marked at the myxedematous level   At the normal level, the only effect was a toxic one

The appearance and disappearance of the clinical improvement lagged considerably behind the rise and fall in basal metabolism when the injection was given at the myxedematous level

The time occupied by the ascending portions of the curves varied from 4 to 6 days, and appeared to be approximately the same at the normal level as at the myxedematous level

The slope of the descending portions of our basal metabolism curves following the injections at the lowest levels of metabolism, does not support the hypothesis of Boothby et al that the reaction is a monomolecular one

CONCLUSIONS

The level of the basal metabolic rate at the time of the intravenous injection of a given dose of thyroxin into a myxedematous patient, has a marked influence upon the amount of excess heat produced and upon the intensity of both the unpleasant and the beneficial clinical effects

The multiplicity of unmeasured factors involved in the reaction produced by thyroxin makes it impossible at present to reduce the effect to accurate mathematical terms   The most important of these factors appear to be the amount of injected thyroxin that is effective, the nature of the chemical changes produced by thyroxin, and the time and rate at which thyroxin disappears from the reaction—a factor which may not be accurately gauged by changes in the basal metabolism

The clinical condition of the patient is just as important as the basal metabolic rate in determining the proper maintenance dose of thyroid for a patient with myxedema

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THE ACTION OF SODIUM CHLORIDE, AMMONIUM CHLO-RIDE, AND SODIUM BICARBONATE ON THE TOTAL ACID BASE BALANCE OF A CASE OF CHRONIC NEPHRITIS WITH EDEMA

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INTRODUCTION

The steps in the clarification of the pathological physiology associated with the edema in the nephrotic type of chronic nephritis form an interesting and as yet unfinished chapter in modern medicine In 1902 Widal (1) pointed out that the failure to excrete water is associated with a difficulty in excreting sodium chloride and advocated a low salt diet In 1909 Blum (2) amplified this by showing that it is only the sodium ion which the kidney has difficulty in excreting In 1911 Meyer and Cohn (3) observed the diuretic action of calcium chloride in infants, and in 1918 Schultz (4) used this drug in treating war nephritis Blum, Aubel, and Hausknecht (5) in 1922 thought that the liberation of edema following the administration of calcium chloride is the result of an antagonism between calcium and sodium which facilitates the excretion of sodium A year later Haldane, Hill, and Luck (6) reported the production of an acidosis by the ingestion of calcium chloride and attributed its diuretic action to this In the same year Gamble, Ross, and Tisdall (7) showed that this acid effect is due to a greater absorption of the chlorine ion than of the calcium ion In the meantime, Haldane (8) in 1921 had demonstrated that the ingestion of ammonium chloride produces an acidosis This he attributed to the transformation of the ammonia into urea, which leaves the acid radicle chlorine to unite with body base In

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1923, Keith, Barrier, and Whelan (9) drew attention to the diuretic action of ammonium chloride in nephritis with edema   Finally in 1925, Gamble, Blackfan, and Hamilton (10) showed that calcium chloride and ammonium chloride bring about a diuresis by producing an acidosis   They pointed out that in order to excrete the chlorine ion there must be a corresponding increase in the titratable acidity of the urine and in the excretions of ammonia and total base   The first two of these factors do not increase sufficiently to take care of the increased chlorine excretion, and therefore base is withdrawn from the body fluids   This base they found to be sodium and potassium   With the increased excretion of base, the water held by the base in the body is liberated, and diuresis results   This excellent paper practically limits the possibilities for clarification in this direction but leaves two things to be explained   What is the cause of the original difficulty in excreting base?   Granted that acid salts act by making necessary an increased excretion of base to prevent severe acidosis, what is the exact physiological means by which they accomplish this?   In other words, we cannot be content with the explanation that the body has to excrete base when an acid salt is taken   We should like to know just how this is brought about

An entirely different approach to this question starts with the work of Epstein (11) (12) who emphasized the low albumin of the serum, the low protein content of edema and ascitic fluid, and the high fat content of the blood as indicated by the high cholesterol   He believed that the hypoproteinemia is the basic disorder and directed his treatment toward the relief of this   Salvesen and Linder (13) in 1923 showed that with the lowered serum protein in nephritis there is a corresponding lowered serum calcium, and that the calcium content of various body fluids bears a definite relation to the protein content   Recently Blackfan and Hamilton (14) have made what is probably a very significant contribution when they demonstrated a low concentration of total base in the serum of children suffering with nephrosis

In summary, we have a condition associated with massive albuminuria, a resulting low serum albumin, a low serum calcium dependent on the low serum protein, a low serum total base possibly also dependent on the low serum protein, a difficulty in excreting base in the urine, a resulting accumulation of base in the tissues, a retention of

water to keep the relation of base to water in the body fluids constant, and hence edema

The present investigation was undertaken in order to test the validity of the above concept, and to discover, if possible, additional relations

A patient with relatively mild, almost stationary, and essentially uncomplicated "nephrosis" was selected who was willing to coöperate in this rather tedious investigation   The details of the clinical history are appended   In brief, she was an adult woman with massive albuminuria, marked edema, low serum albumin, high blood cholesterol, normal renal function as shown by the excretion of phenolsulphophthalein, normal blood non-protein nitrogen, normal urinary sediment, and normal blood pressure.

She was transferred to the Metabolism Ward where she was studied for twelve three-day metabolism periods   During this entire time (36 days) she received a carefully weighed diet[2] containing exactly the same articles of food every day   Likewise, the same amount of fluid was administered every day at the same hours   She was allowed out of bed for exactly the same amount of time every day   The urine and feces were collected in three day periods for analysis   Venous blood for analysis was withdrawn under oil at frequent intervals   All determinations were made on serum

The urine was analyzed for what we have termed "titratable acidity minus CO_2," ammonia, total base, calcium, phosphorus, sulphates, chlorine, protein, nitrogen, and non protein nitrogen, the feces for calcium, total base, and phosphorus, the serum for total base, calcium, protein, albumin, globulin, CO_2 combining power, chlorine, and phosphorus.   The methods employed in the collection of the excreta, the preparation of the diet, and the analyses of the serum, urine, and feces have been given in a previous paper (15)   The titratable acidity minus CO_2 and the ammonia were analyzed daily   The value of the former expression was obtained by adding a known amount of acid to the urine, shaking until the CO_2 was entirely driven off, and then

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[2] The details of the diet are appended.

titrating back to a pH of 7 35   The amount of alkali minus the amount of acid added equals the "titratable acidity minus CO_2" In an alkaline urine this may become a negative expression

The investigation consisted of a sequence of three control periods (9 days), two in which salt was added to the diet, two in which this was replaced by ammonium chloride, one control period, one in which sodium bicarbonate was administered, and finally three control periods

<div align="center">RESULTS</div>

<div align="center">*A   Total base[4] metabolism (See table 1 and chart 1)*</div>

In chart 1 there are given graphically the data for the total base exchange, the output of urine, and the body weight   Since body fluids contain approximately the base equivalent of 150 cc of N/10 base per 100 cc (16), the retention of 150 cc of N/10 base should be associated with the retention of 100 cc of fluid and a gain in weight of 100 grams   This of course is true, provided the patient is in nitrogen balance   (An insignificant error enters into this reasoning due to the deposit of base as calcium in the bones)   Therefore, in chart 1 the scale for the weight is chosen such that 1000 grams on the weight scale is equivalent to 1000 cc of N/10 base on the base scale   Likewise, the urine is charted on a scale of the same size as the weight

An examination of chart 1 shows that, during control periods 1, 2, and 3 on a low base intake, the patient was approximately in total base equilibrium and that the weight and urinary output were almost stationary   During periods 4 and 5, in spite of a marked increase in the total base intake, due to the ingested sodium chloride, there was no increase in the total base output except for a slight rise in the urinary base in period 5   The entire added base was retained in the body, the body weight rose and the amount of urine decreased   During periods 6 and 7, when the chlorine was added in the form of ammonium chloride instead of sodium chloride, there was a marked increase in total base excretion with a negative total base balance and a corresponding increase in output of urine and decrease in body weight   In control period 8 there was a return to equilibrium   During period 9,

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[4] By total base is meant the sum of calcium, magnesium, sodium, and potassium expressed in cubic centimeters of N/10

when the base was administered in the form of sodium bicarbonate in place of sodium chloride, there was a marked rise in the total base
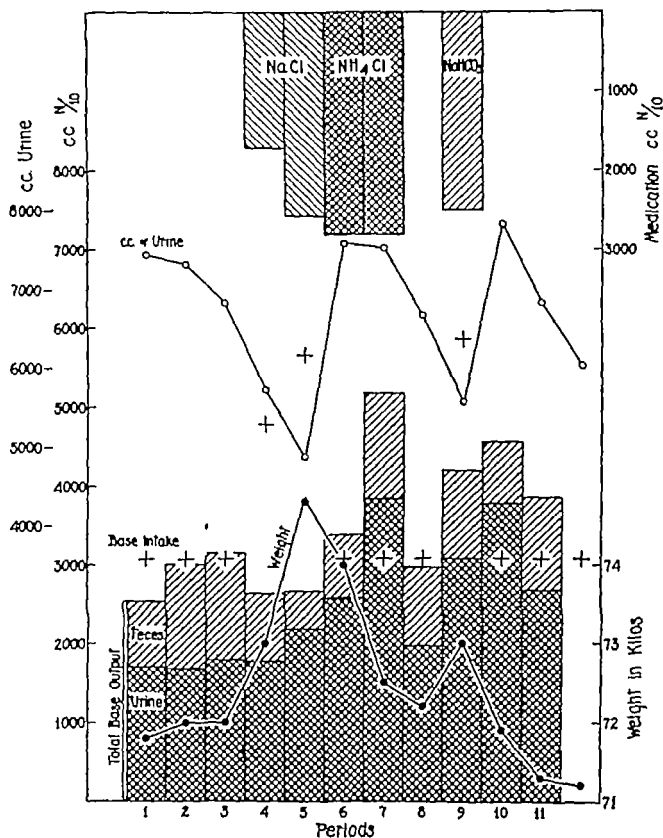
CHART 1 GRAPHIC REPRESENTATION OF DATA FROM TABLE 1

excretion, not, however, equal to the increased total base intake Corresponding to the retained base, there was a decrease in output of

urine and an increase in weight In control period 10, the increased base excretion continued During period 11, equilibrium was again reached From the last two columns of table 1 it will be noted that the change in weight from period to period corresponded extremely well with the change in weight as calculated from the total base balance

TABLE 1

The data for the total base balance, output of urine and body weight for the 36 days of observation

It will be noted that the actual change in weight from period to period corresponds extremely well with the change in weight as calculated from the total base balance

| Period | Total base | | | | | Urine | Weight | Medication | Gain in weight | Gain in weight calculated from base balance |
	Intake	Urine	Feces	Total output	Balance					
	cc of N/10	cc of N/10	cc. of N/10	cc of N/10	cc of N/10	cc	kgm	cc of N/10	kgm	kgm
1	3,087	1,700	842	2,542	+545	7,445	71 8		+0 2	+0 3
2	3,087	1,686	1,327	3,013	+74	7,320	72 0		+0 2	0 0
3	3,087	1,795	1,358	3,153	−66	6,825	72 0		0 0	0 0
4	4,797	1,782	852	2,634	+2,163	5,725	73 0	1,710 of NaCl	+1 0	+1 4
5	5,652	2,178	483	2,661	+2,991	4,880	74 8	2,565 of NaCl	+1 8	+2 0
6	3,087	2,569	816	3,385	−298	7,590	74 0	2,800 of NH4Cl	−0 8	−0 2
7	3,087	3,890	1,289	5,179	−2,092	7,530	72 5	2,800 of NH4Cl	−1 5	−1 4
8	3,087	1,966	1,005	2,971	+116	6,665	72 2		−0 3	+0 1
9	5,587	3,083	1,110	4,193	+1,394	5,570	73 0	2,500 of NaHCO₃	+0 8	+0 9
10	3,087	3,775	784	4,559	−1,472	7,820	71 9		−1 1	−1 0
11	3,087	2,675	1,179	3,854	−767	6,820	71 3		−0 6	−0 5
12	3,087		942			6,020	71 2		−0 1	

From the data thus far, the following points seem significant

1 Gains and losses of edema fluid could be approximately predicted from the total base balances on the supposition that 100 cc of tissue fluids are equivalent to 150 cc of N/10 base

2 Addition of the neutral salt sodium chloride for a period of six days resulted in an almost quantitative retention of the added base

3 In spite of inability to excrete base, the absorption of sodium chloride was quantitative

4 Addition of the acid-producing salt, ammonium chloride, increased the total base excretion in the urine and this increase was

more marked during the second three days than during the first three days   This effect stopped immediately on cessation of the drug

5 Addition to the diet of the alkaline salt, sodium bicarbonate, increased the total base excretion but not sufficiently to offset the increased base intake   This increased excretion continued and even increased during the first control period following the sodium bicarbonate period

TABLE 2

Data showing the calcium and phosphorus balances

Period	Calcium				Phosphorus				Medication
	Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance	
	cc. of N/10	*cc. of N/10*	*cc. of N/10*	*cc. of N/10*	*cc. of N/10*	*cc. of N/10*	*cc. of N/10*	*cc. of N/10*	*cc. of N/10*
1	154	17	208	−71	1 885	1,275	496	+114	
2	154	18	250	−114	1,885	1,022	774	+89	
3	154	36	228	−110	1,885	990	703	+192	
4	154	19	153	−18	1,885	2,270	502	−887	1,710 of NaCl
5	154	28	129	−3	1 885	1,179	624	+82	2,565 of NaCl
6	154	77	146	−69	1 885	1 445	504	−64	2,800 of NH₄Cl
7	154	110	195	−151	1 885	5 120	611	−3846	2,800 of NH₄Cl
8	154	81	147	−74	1,885	712	425	+748	
9	154	27	171	−44	1 885	922	505	+458	2,500 of NaHCO₃
10	154	45	113	−4	1 885	900	433	+552	
11	154	32	202	−80	1 885	1,119	596	+170	
12	154	10	157	−13	1 885		578		

B *Calcium and phosphorus metabolism*

It will next be of interest to note the calcium metabolism and to see whether this component of the total base metabolism behaves in any way similar to the total base as a whole   The basic diet was low in calcium and similar to that used in a study of the calcium excretion of normal individuals (17)   In that study of thirteen normal men with an average calcium intake of 165 cc. of N/10 calcium per three-day period, there was an average output in the urine of 85 cc. of N/10 calcium, in the feces of 300 cc of N/10 calcium, making an average negative calcium balance per three day period of 220 cc of N/10 calcium

In control periods 1, 2, and 3 (table 2) it will be noted that the calcium excretion in the urine was strikingly small and that the negative calcium balance on a low calcium diet was likewise small [5] The low calcium excretion in nephrosis has been noted by Scriver (18) It will be further noted that ammonium chloride increased the urinary excretion of calcium (periods 6 and 7) whereas Scriver found that parathormone was ineffective in accomplishing this in nephrosis (18)

The ratio of the urinary to the fecal phosphorus excretion suggests that there was no difficulty in excreting phosphorus through the kidneys The increased phosphorus excretion in the urine, as a result of ammonium chloride, was far out of proportion to the increased calcium excretion, if one were assuming that these were derived from tertiary calcium phosphate from the bones There was no corresponding breakdown of nitrogen to account for the phosphorus deficit (v infra) One must conclude that this phosphorus came from phosphorus held in tissue fluids The increased phosphorus excretion resulting from the ingestion of hydrochloric acid has been discussed by Fitz, Alsberg, and Henderson (20)

The following points from the calcium and phosphorus data seem significant

6 The urinary calcium excretion was very much lower than that of normal individuals on a similar régime and there was no corresponding increased fecal calcium excretion

7 Ammonium chloride increased the urinary calcium and phosphorus excretions without affecting that in the feces The increase in the urinary phosphorus was out of proportion to the increase in the urinary calcium

C Nitrogen metabolism

Before one can calculate gains in weight as gains in edema fluid (v supra), it is necessary to know that the subject was in nitrogen equilibrium Therefore, table 3 and chart 2 have been constructed It will be noted that on a nitrogen intake of 25 9 grams per period (54 grams of protein a day) the patient was in nitrogen balance

[5] The low urinary excretion in chronic nephritis with edema has been confirmed in two other unpublished cases studied at the Johns Hopkins Hospital in conjunction with Dr Read Ellsworth

This was true in spite of rather large losses of protein in the urine
The protein losses in the urine in this case were smaller than one often
finds in similar cases   The nitrogen from the ammonium chloride
in periods 6 and 7 appeared almost quantitatively as ammonia in the
urine   From this it would appear that the acidosis produced by the
conversion of the ammonia into urea, when ammonium chloride is
absorbed, may be later compensated for by the reconversion of urea
into ammonia when the chlorine is excreted

TABLE 3

Data showing nitrogen metabolism

Period	Intake	Non-protein nitrogen in urine	Protein nitrogen in urine	NH₃ nitrogen in urine	Feces†	Balance	Medication
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*cc. of N/10*
1	25 9	18 65	4 86	0 39	2 59	−0 20	
2	25 9	20 61	6 65	0 82	2 59	−3 95	
3	25 9	18 90	4 80	0 76	2 59	−0 39	
4	25 9	17 83	4 50	0 93	2 59	+0 98	1,710 of NaCl
5	25 9	16 64	3 20	0 95	2 59	+3 47	2,565 of NaCl
6	29 8	21 47	4 59	2 09	2 98	+0 74	2,800 of NH₄Cl
7	29 8	21 82	4 22	5 30	2 98	+0 78	2,800 of NH₄Cl
8	25 9	17 54	5 35	3 91	2 59	+0 42	
9	25 9	16 28	3 02	1 18	2 59	+4 01	2,500 of NaHCO₃
10	25 9	20 80	5 20	0 88	2 59	−2 69	
11	25 9	20 83	4 91	1 25	2 59	−2 43	
12	25 9			1 58	2 59	−1 29	

* This is inclusive of ammonia nitrogen which is represented in a separate column
†Fecal nitrogen taken as 10 per cent of nitrogen intake

From this study of the nitrogen metabolism, the following points
seem important

8 In spite of protein loss in the urine, the patient remained in
nitrogen equilibrium on 54 grams of protein a day

9 When ammonium chloride was ingested, the ammonia excre-
tion in the urine rose proportionately to the increased intake

D Total acid-base balance of urine

Table 4 and chart 3 have been constructed in order to study the acid-
base balance of the urine during the varying conditions of this inves-

tigation   In this we have followed the line of approach used by Gamble (19) with a slight modification   This author, by determining
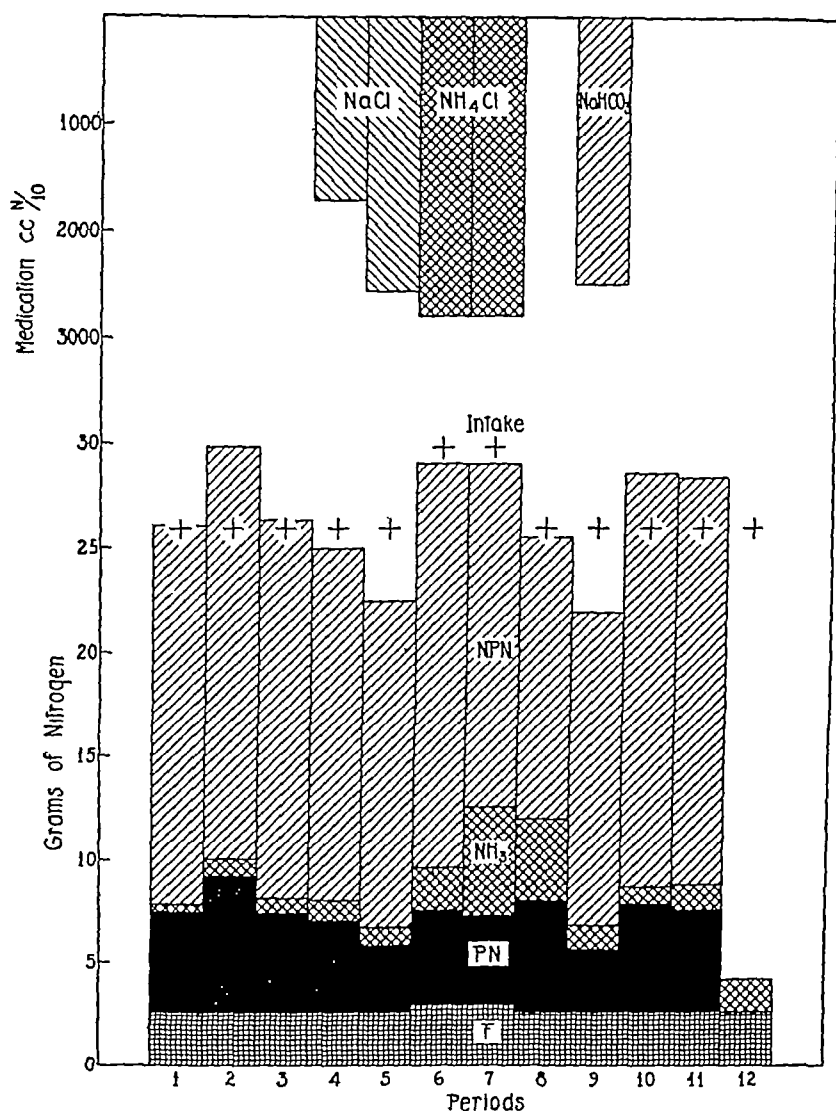
CHART 2   GRAPHIC REPRESENTATION OF DATA SHOWN IN TABLE 3

the titratable acidity of the urine (= base necessary to bring pH of urine to 7 35), was able to construct tables and charts in which the

cations of the urine in one column were balanced by the anions in another Thus, the sum of total base, ammonia, and titratable acidity, all expressed in cubic centimeters of N/10 equals the sum of the chlorides, sulphates, phosphates, organic acid and carbonic acid also expressed in cubic centimeters of N/10 except that a factor has to be

TABLE 4

Data for acid-base balance of the urine during the varying conditions of this investigation

Period	Titratable acidity minus CO₂	Cations in urine			Base bound by anions of urine						Medication	Chlorine intake
		NH₃	Total base	Sum of cations	Cl	SO₄	P at pH 7.35	Protein at pH 7.35	Base bound by determined anion	Organic acid		
	cc of N/10	cc of N/10	cc of N/10	cc of N/10	cc of N/10	cc of N/10	cc of N/10	cc of N/10	cc of N/10	cc of N/10	cc. of N/10	cc of N/10
1	41	279	1 700	2 020	104	404	773	53	1 334	686		570
2	−21	589	1 686	2 254	106	470	620	72	1 268	986		570
3	−47	543	1,795	2 291	188	463	595	52	1 298	993		570
4	163	667	1 782	2 612	313	410	1,370	49	2,142	470	1 710 of NaCl	2 370
5	400	683	2 178	3 261	1,008	358	709	35	2,110	1,151	2 565 of NaCl	3,260
6	652	1,492	2,569	4 713	3,280	451	864	50	4 645	68	2 800 of NH₄Cl	3 370
7	684	3 785	3,890	8,359	4,813	371	3,070	46	8 300	59	2,800 of NH₄Cl	3 370
8	468	2,787	1,966	5 211	2 058	358	428	58	2,902	2,309		570
9	−1 008	843	3,083	2,918	546	318	554	33	1 451	1,467	2 500 of NaHCO₃	570
10	−1 017	630	3,775	3 388	716	477	540	56	1 789	1,599		570
11	−57	897	2 675	3,515	848	444	720	53	2,065	1,450		570
12	202	1 134				470						570

introduced for the phosphates, which at the pH of 7 35 bind only 1 8 equivalents of base rather than 3

We have modified this scheme in that both our cation and our anion columns are shorter by the carbonic acid value, i e , on the cation side our third value is the "titratable acidity minus CO₂" instead of the titratable acidity, and on the anion side we have omitted the
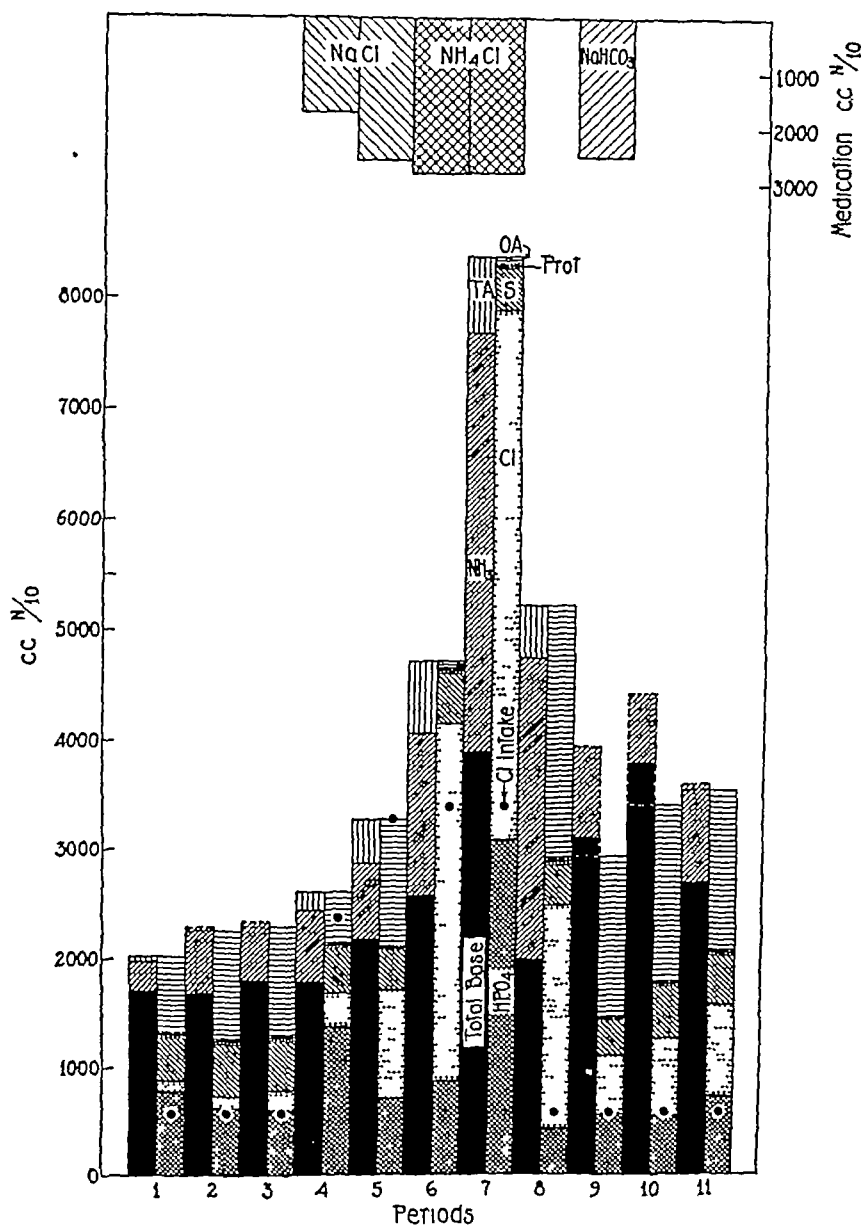
CHART 3 GRAPHIC REPRESENTATION OF DATA SHOWN IN TABLE 4

carbonic acid   We have not estimated the organic acids but have
assumed this to be represented by the difference between the cation
column and the sum of the other anions   Such values for the organic
acids contain the errors of all the determinations   In addition, be-
cause of the large amount of protein in the urine, we have attempted
to ascertain the base-binding value of this at pH 7 35 by using the
formula of Van Slyke, Wu and McLean (21) for base bound by serum
protein,

$$BP = 0.68\,P \quad (pH - 4.80)$$

in which BP and P, represent respectively cubic centimeters of $N/10$
base per 100 cc of serum combined with protein, and per cent of protein
in the serum   We appreciate that any value for base bound by urine
protein thus obtained is extremely inaccurate, especially as the pro-
tein is mostly albumin rather than a mixture of albumin and globulin

It is of interest to compare the chlorine excretion in the urine with
the chlorine intake (see last column in table 4)   During the control
periods 1, 2 and 3 the urinary excretion of chlorine was very low, about
$\frac{1}{4}$ of the intake   During the sodium chloride periods the urinary
excretion went up, but very much less than the intake   This does
not show an inability to excrete chlorine but merely represents the
retention of chlorine necessary for offsetting the retained base   In the
ammonium chloride periods, the urinary excretion of chlorine exceeded
the intake, which was to be expected   The excess represents the
chlorine held by the base in the edema fluid   The increased chlorine
excretion continued into control period 8   During the sodium bicar-
bonate period the urinary chlorine was again reduced, probably
because chlorine was again withheld to offset the withheld base.
During control periods 10 and 11 the excretion again rose above the
intake concomitant with the loss of edema fluid

In chart 3, during control periods 1, 2, and 3 it will be noted that
the "titratable acidity minus CO_2" was small or negative   During
the sodium chloride period the anion columns were increased due to a
rise in the chlorine and phosphorus excretion   This was compen-
sated for in the cation column by a rise in the "titratable acidity
minus Co_2" and a rise in the ammonia   During the ammonium
chloride periods the anion columns were very much increased due to

the marked increase in the chlorine and phosphorus excretions
This was compensated for on the cation side by a marked increase
in all components   In control period 8 the findings still showed the
effect of period 7, but it will be noted that the total base excretion
was the first of the cation components to return to its previous level
This rather suggests that total base excretion in response to an acid
is a last line of defense and only takes place after the ammonia mech-
anism and the titratable acidity have been taxed to their limits
During the sodium bicarbonate period, the "titratable acidity minus
CO_2" became a negative quantity due to the large amount of CO_2
In this period and the following period it would appear that anions
were in demand to neutralize cations which were being excreted in
excess, whereas in the ammonium chloride periods, the exact opposite
was the case   In other words, the increased excretion of base as a
result of ammonium chloride ingestion may be thought of as an effort
on the body's part to ward off fatal acidosis, whereas the increased
excretion of base as a result of sodium bicarbonate is an indica-
tion that the kidneys in this case can excrete base under certain
conditions

The figures for the organic acid excretions are of interest, although
it must be repeated that they contain all the errors of this investiga-
tion   They showed little of interest during the first five periods
During the ammonium chloride period there was a marked decrease
in organic salt excretion, possibly because base was at a premium
and it was more important to excrete the mineral acid chlorine
The increased organic acid excretion in the following control period
may represent this stored organic acid   During the sodium bicar-
bonate period and the period following, the organic acid excretions
were high, possibly because anions were in demand for excretion
with the base

From table 4 and chart 3 the following observations seem significant

10   Provided that it was not being withheld to offset the retained
fixed base, chlorine was readily excreted

11   When additional sodium chloride was ingested, the sodium was
entirely retained, part of the chlorine was withheld to offset the
sodium, the rest of the chlorine was excreted in the urine with a re-
sulting rise in the titratable acidity and the ammonium excretion

12 Addition of ammonium chloride caused a marked rise in the chlorine and phosphorus excretion and this was offset by a rise in all the cation components of the urine

13 The increased excretion of fixed base after ingestion of acid tended to occur only after other mechanisms for the neutralization of acids had been thoroughly taxed

14 The increased excretion of fixed base after alkali ingestion apparently depended on a primary increased ability to excrete base

TABLE 5

Data for serum electrolytes obtained at various times during this investigation

Period	Day of period	Calcium		Base bound by		Chlorine	Protein	Base bound by protein	Base bound by total determined acid	Total base	Organic acid	Total base excretion in urine in corresponding period
				Phosphate	CO_2							
		mgm.	cc. of $N/10$	cc of $N/10$	cc of $N/10$	cc. of $N/10$	grams per 100 cc.	cc of $N/10$	cc of $N/10$	cc of $N/10$	cc of $N/10$	cc of $N/10$
2	2						4 28	7 43		144 5		1,686
3	3	8 65	4 32	2 48						147 0		1 795
5	3	8 15	4 07	2 28	27 3	107 5	3 58	6 20	143 3	157 5	14 2	2 178
7	2	8 80	4 40	2 36	24 9	112 0	4 55	7 90	147 2	158 0	10 8	3 890
8	2	8 50	4 25	2 32	28 0	106 0	4 20	7 30	143 6	149 0	5 4	1 966
9	3	8 65	4 32	2 50	31 4	103 4	4 30	7 46	144 8	158 0	13 2	3,083
11	3	8 30	4 15	2 02	26 0	105 8	3 80	6 60	140 4	148 0	7 6	2 675

15 Organic acids apparently were retained in the body when base was at a premium and were excreted in excess when acid was at a premium

E Serum electrolytes

The serum findings are shown in table 5 and chart 4 (q v)   All values are on serum   In determining the base bound by protein we used the formula of Van Slyke, Wu, and McLean (v supra) and assumed a pH of 7 35   The objections to this formula have been discussed by Peters, Bulger, Eisenman and Lee (22), but it offers an approximation which is probably satisfactory for comparative pur-

poses   Base as $BHCO_3$ was calculated by the equation of Peters, Bulger, Eisenman and Lee (22)

$$B = \frac{CO_2 - 2\,85}{2\,24}$$

where 2 85 volumes per cent is equal to the amount of CO_2 dissolved in serum at 40 mm of CO_2 tension and 38°C   The phosphorus in milligrams per 100 cc was reduced to cc of N/10 per 100 cc by the
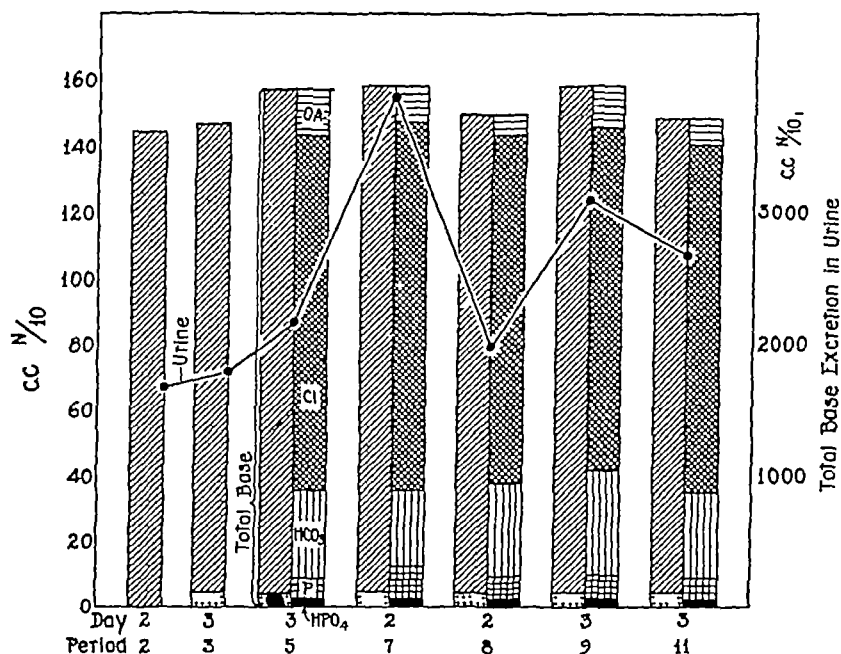
CHART 4 GRAPHIC REPRESENTATION OF DATA SHOWN IN TABLE 5

factor 1 8/3 1, accepting L J Henderson's (23) estimate of the proportion of primary and secondary phosphate in the blood   The difference between the sum of the base bound by the determined acid and the total base we have termed "organic" acid, but sulphates are included also as a component of this value

The serum calcium was definitely low by about 1 5 mgm without any corresponding rise in serum phosphorus   This is undoubtedly due to the low serum protein as pointed out by Salvesen (13)   The serum total base was likewise low during control periods 2, 3, 8, and

11   This supports the work of Blackfan and Hamilton (14). These authors give the normal range of serum total base as 154 to 161 cc. of N/10 base   It is of interest that this lowered base value rose to normal during the latter part of the sodium chloride ingestion, during the ammonium chloride period, and during the sodium bicarbonate period   When the total base value of the serum is compared with the total base excretion, it will be observed that at least in these few instances the serum total base rose as the excretion increased   During the ammonium chloride periods the CO₂ fell and the chlorides rose as was to be expected   During the sodium bicarbonate period the opposite was true

TABLE 6

Data for spinal fluid electrolytes

	Calcium	Base bound by phosphorus	Base bound by CO₂	Chlorine	Base bound by protein	Base bound by total deter mined acid	Total base	Organic acid
	cc of N/10	cc of N/10	cc. of N/10	cc of N/10	cc of N/10	cc of N/10	cc of N/10	cc of N/10
Normal serum water*	5 10	2 03	25 4	105 0	12 3	144 7	159	14 3
Serum water of patient	4 45	2 42	29 2	111 0	7 6	150 2	156	5 8
Spinal fluid	2 45	1 00	23 2	127 5	0 02	121 7	161	9 3

* Values calculated from normal values given by Blackfan and Hamilton (14)

The following points here seem important

16   The blood serum before medication differed from the normal in that the total protein was low, the total base was low, and the calcium was low

17   Ammonium chloride, sodium bicarbonate and sodium chloride ingestion raised the serum total base

18   The total base excretion increased in the urine when the serum total base was raised

F   Spinal fluid electrolytes

A lumbar puncture was done during period 8, and it is of interest to compare the values for electrolytes found in the spinal fluid with those in the blood serum withdrawn at the same time   It is first

necessary to reduce the serum values to their values per 100 cc of serum water   The formula of Van Slyke, Wu, and McLean (21) has been used, according to which the grams of water in 1 liter of serum equal $990 - 0 8P$, where P is the grams of protein per liter of serum

The spinal fluid findings are perfectly consistent with normal values   The most important difference between spinal fluid and serum is the small amount of protein in the spinal fluid   This is compensated for by increased chlorides on the anion side   The calcium in spinal fluid is likewise low due to the low protein   As the serum protein is lowered in chronic nephritis with edema, it would appear that the serum findings approach the spinal fluid findings as a limit   The spinal fluid phosphorus, as has been repeatedly shown in this laboratory[6], is lower than serum phosphorus, apparently in order to keep the same ratio of Ca to P found in the blood and in the bones

The following observations then seem important

19 The spinal fluid electrolytes were normal

20 The serum findings differed from normal serum findings very much as spinal fluid findings differ from serum findings only to a lesser degree

21 The ratio of calcium to phosphorus in the spinal fluid was approximately the same as that in the blood, a relationship which we have observed in normal subjects

It will be unnecessary to discuss all the findings here as this would lead to considerable repetition   Three points however seem worthy of emphasis

The pathological physiology of the fixed base metabolism in chronic nephritis with edema has many points in common with that of the calcium metabolism   Thus the urinary excretion of both is low without there being any compensatory increased fecal excretion, both are low in the blood, possibly accounting for the low urinary excretion, and ammonium chloride ingestion increases the urinary excretion of both and probably raises the serum values of each

It seems fairly well established by the work of Blackfan and Hamilton (14) that the serum total base is lowered in chronic edema   This is corroborated in the present study   It is suggested from this study

[6] Unpublished data

that agents which tend to raise the lowered serum base value toward normal increase the total base excretion in the urine   In a recent contribution by Linder (24) it is of interest that one of the two cases of hydremic nephritis (case VII) had an initial total base in the serum of 135 cc. of N/10 per 100 cc., which rose to 145 cc. of N/10 under hydrochloric acid therapy   All this of course suggests that the low output of base is due to the serum base being below the threshold of kidney excretion

The most surprising thing to us in this investigation was the response to sodium bicarbonate ingestion   It is true that the patient did become more edematous but much less so than we had expected   It would seem that the ingestion of large amounts of sodium bicarbonate likewise raises the serum base to the normal threshold of kidney excretion with a resulting increased base excretion   Our findings support the recent work of Osman (25) (26) who found that on the administration of very large doses of alkali to such patients, there was at first an increase of edema until a critical point was reached when there occurred an extraordinary diuresis

SUMMARY

The total acid base balance of the urine, the fixed base, calcium and phosphorus balances of the urine and feces, frequent determinations of the serum electrolytes, and one simultaneous determination of the spinal fluid and blood serum electrolytes, have been made on a mild case of chronic nephritis with edema during twelve three day periods on a constant intake in order to determine the effect of sodium chloride, ammonium chloride, and sodium bicarbonate ingestion

APPENDIX

The patient was a 35 year old married German woman who was admitted to the Massachusetts General Hospital on February 24, 1927 complaining of generalized edema.

The past history was unimportant except for measles and chicken pox in childhood, an attack of tonsillitis at the age of 15, and since then occasional sore throats.

The present illness started fifteen years ago with slight swelling of the ankles at the end of the day   This swelling gradually became more extensive.   Six months before admission she noted swelling of the face

Physical examination showed massive edema of the legs and slight edema over

the sacrum   The blood pressure was 130/80   The heart was within normal limits

The urine examination showed a large amount of albumin and a normal sediment. The specific gravity varied between 1 005 and 1 015   The phenolsulphophthalein test showed a normal excretion of the dye   The red blood cells were 4,800,000 and the white cells 8,600 per cubic millimeter   The blood non-protein nitrogen was 25 mgm per 100 cc   The serum protein was 4 28 grams per 100 cc with a serum albumin of 2 3 grams per 100 cc   The blood cholesterol was 222 mgm per 100 cc   The basal metabolism was minus 3 per cent

The impression was that this patient was suffering from a rather mild and quite stationary form of chronic nephritis of the nephrotic type

A letter from the patient dated June 19, 1928 states that her condition has remained stationary during the intervening year

Diet

Food substance	Weight	Protein	Fat	Carbo-hydrate
	grams			
Bread, salt free	100	8 0	2 1	53 2
Butter fat	40		40 0	
Sugar	30			30 0
Apple (raw)	200	0 6	1 0	27 0
Orange juice	80			8 64
Banana	100	1 1	0 6	21 0
Steak	150	31 95	12 0	
Potato	100	1 9	0 1	18 1
Corn	100	2 8	1 2	19 0
Tomatoes (raw)	100	1 2	0 2	4 0
Chicken (white)	30	6 45	0 75	
	cc			
Coffee	200			
Tea	400			
Total		54 00	57 95	180 94

Total calories, 1461

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PROCEEDINGS OF THE TWENTY-FIRST ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N J, MAY 6, 1929

Blood Vessels of Non-Inflammatory Origin in Human Heart Valves   By Albert W. Bromer, and Louise J. Zschiesche, (by invitation) and Joseph T. Wearn, Boston, Mass

By a method of procedure not used heretofore, blood vessels have been demonstrated in one or more valves in 74 of a series of 100 human hearts   No heart with the clinical diagnosis of endocarditis or rheumatic fever has been included A small percentage of the individuals were sufferers of arterio-sclerotic heart disease

After as much air as possible has been drawn from the coronary system, Weber's or Higgins' India ink, diluted with an equal amount of distilled water, is injected into the coronary arteries under a pressure of 220 mm. of mercury   During the injection procedure, the heart is gently massaged in a tank of physiological saline solution at a temperature of 38°C   Inability to maintain an air tight system has probably been a source of error not infrequently

The valve leaflets of all hearts injected have been rendered transparent by the Spalteholz method for microscopic examination   The mitral valve has presented vascularization in 58 per cent of the hearts injected, the tricuspid valve in 43 per cent, the pulmonary valve in 25 per cent, and the aortic valve in 5 per cent.   Leaflets of two or more valves have been successfully injected in 39 per cent of the total number

The presence of vessels in the valves seemingly bears no relationship to age, the number of vascularized valves of the higher decades being approximately the same as for each of the earliest decades

The vascularization of the aortic leaflet of the mitral valve is characterized by two or more vessels of goodly calibre descending in a sweeping looping manner from the auriculo-ventricular juncton toward the line of closure, where they join in a meshwork of anastomoses   Occasionally vascular twigs pass to the free margin of the leaflet.   In some specimens vessels run upward in the chordae tendineae, stopping short of the free margin of the leaflet, many looping about to return to the papillary muscle   Occasionally, the chordae vessels form anastomoses with vessels in the basal third of the leaflet.   Not infrequently, only one of the large descending vessels may be present, injection of the others having possibly failed through blockage with air, blood cells or a large carbon particle   The posterior mitral leaflet is supplied by three or more large vessels descending with loops, arborizations and anastomoses   These vessels lie close to the superior surface of

the leaflet   In the non-cleared specimens they are not visible on the under side
Histologically, the larger vessels show true arterial structure

Tricuspid leaflet injections are characterized by extremely fine vascular twigs
extending downward 4 to 8 mm   As in the mitral valve, the looping and inter-
lacing arborizations are most impressive   Vessels ending blindly indicate an in-
complete injection

The pulmonary cusp vascularization, as a rule, is limited to the lower one-half
or two-thirds of the membranous portion, with fine arterioles and capillaries ex-
tending into the cusps proper from along the line of attachment   The filmy tex-
ture renders injection most difficult because of collapse or kinking of the delicate
vessels

The capillary architecture of the aortic cusps is approximately the same as that
of the pulmonary cusps, with a rich vascular bed in the commissures   Vessels
occasionally run along the line of closure to the corpus Arantii   Studies are now
in progress to determine whether the relatively small percentage of successful
aortic cusp injections may be explained in many instances by the main blood sup-
ply being derived from the vasa vasorum rather than from the immediate coronary
vessels

*Experiments on the Patency of the Blood Vessels of Nephritic Kidneys Obtained
at Autopsy*   By J M HAYMAN, JR , Philadelphia, Pa

One of the first questions which arise in the study of the physiology of the dis-
eased kidney concerns the capacity of its vessels to permit a normal blood flow
These experiments aimed to distinguish those types of kidney damage in which a
mechanical, anatomic obstruction to blood flow exists from those in which the
structural condition of the vessels might be regarded as permitting a normal blood
flow

Kidneys obtained at autopsy were perfused with Ringer's solution at 100, 150
and 200 mm Hg pressure and the perfusate collected from the renal vein   The
results have been compared with roentgenograms of the arterial tree after injec-
tion of bismuth and with histological sections

Since large kidneys permit a greater volume of perfusate per minute than small
ones, all data were reduced to flow per gram of kidney per minute   This of course
introduces a disturbing factor in kidneys edematous from disease, but this is off-
set by the advantages of the method in comparing perfusability of different kidneys

The volume of perfusate per gram of kidney in 18 normal adult kidneys varied
from 1 2 to 2 5 cc per minute at 100 mm Hg perfusion pressure, from 2 1 to 4 3
cc per minute at 150, and from 2 5 to 5 5 at 200 mm pressure   There was no
detectable relation between volume of perfusate per gram of kidney and age

Kidneys showing purely degenerative changes, the so-called nephroses, allowed
as great perfusion flows as normal kidneys   This group includes a kidney of
pregnancy and one of bichloride poisoning

Kidneys from 9 cases of benign arteriolarsclerosis showed a decrease in volume

of perfusate per gram roughly proportional to the degree of sclerosis   Two of these showed only mild changes histologically and the perfusion flows fell within lower normal range   Four kidneys showed moderately advanced changes histologically   One of these gave a perfusion flow within the lower normal limit, the other three distinctly below it.   The other three kidneys showed advanced changes and the perfusion flow per gram of kidney was greatly reduced in all.   Unfortunately suitable methods are not available for estimating quantitatively the decrease in number of patent glomeruli per gram so that it is uncertain what part decrease in number of perfused units and increased resistance in existing vessels play in the diminished volume of perfusate   Since the relative increase in volume of perfusate with increase in pressure is of the same order in normal and arteriolarsclerotic kidneys it seems probable that decrease in the number of patent glomeruli is the more important factor

Two kidneys showing mild acute diffuse nephritis gave perfusion flows below the normal range   Kidneys from 5 cases of chronic glomerulonephritis and uremia showed a marked reduction of perfusate per gram of kidney at all pressures   The average flow per minute was 0 5 cc at 100 mm pressure, 0 9 at 150, and 1.2 at 200   These flows were of the same order as those found in advanced arteriolarsclerosis

Capillary Blood Flow in Man during Fever [1] By FRANK FREMONT-SMITH, and
        (by invitation) L RAYMOND MORRISON, and ALEXANDER W MAKEPEACE,
        Boston, Mass

Direct microscopic observations of the human nailfold capillaries have been made during the febrile reaction produced by the intravenous injection of typhoid vaccine

Coincident with the onset of the fever there occurs complete stasis in practically every visible capillary   From a normal flow this stasis reaches its climax rapidly, often suddenly, and continues until the fever has nearly reached its height.   During the period of stasis cyanosis occurs

Just before the height of the fever is reached the blood flow in the capillaries begins again, at first slowly, but within a short time becomes enormously rapid with the appearance of capillary pulsation and the disappearance of the cyanosis   The fever now begins to fall.   The rapid flow lasts throughout the period of falling temperature and often for many hours afterwards

This same series of phenomena occurs in malaria and in sodoku (rat bite fever)

Evidence is brought forward to show that the stasis is produced by constriction of the terminal arterioles

The local application of heat prevents the occurrence of the stasis, or abolishes the stasis once it has occurred

At the meeting of this Society in April 1928 one of us (Fremont-Smith, F , Dailey M E and Thomas G W) presented evidence that dilution of the blood

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[1] This investigation was aided by a grant from the Ella Sachs Plotz Foundation

serum and cerebrospinal fluid occurs during the febrile response to intravenous injection of typhoid vaccine, analogous to the dilution seen in many acute infections

We have shown that this dilution of the blood serum occurs as a result of retention of ingested water within the organism   No dilution occurs if water is withheld   Water drinking normally results in prompt diuresis   During experimental typhoid vaccine fever, however, (and also in malarial fever) diuresis is delayed from six to twelve hours or longer

The antidiuretic effect of typhoid vaccine fever begins coincidently with the constriction of the skin arterioles described above   This could be explained by a similar vasoconstriction of the renal vessels   Mendelson, 1883, demonstrated such a vasoconstriction in the kidney during experimental fever in dogs

The influence of this antidiuretic effect of fever upon the development of cardiac and nephritic edema is discussed

The Dynamics of the Circulation in Hypertension, and Its Bearing on Therapy   By
    SOMA WEISS and (by invitation) LAURENCE B ELLIS, Boston, Mass

Few, if any, systematic studies of the circulation in arterial hypertension have been made   As a result, there exists no adequate knowledge of the function of the circulation in this condition, and, therefore, rational therapeutic measures cannot be applied

In an endeavor to throw some light on this subject, a group of patients with arterial hypertension, but without evident cardiac failure or kidney damage, were studied, with the object of discovering wherein the physiology of the circulation in hypertension differs from the normal   The arterial blood pressure was determined by the Riva Rocci method, the arteriolar and capillary blood pressure of the vessels of the skin was estimated with modified Recklinghausen capsules, the venous blood pressure was measured by the method of Moritz and Tabora   The vital capacity of the lungs was obtained with a portable spirometer, the cardiac output per minute and per stroke was ascertained by the carbon-dioxide method, the basal metabolism was obtained with the Tissot method   The velocity of blood flow was tested with the radio-active deposit and the histamine methods, and, finally, the blood volume was determined by the dye method   In a group of 16 patients, nearly all of the above measurements were undertaken repeatedly under basal conditions   In an additional group of 30 patients several of the circulatory functions were studied

These findings indicate that, in essential arterial hypertension, although an abnormally high pressure exists in the arteries and arterioles, the capillary and venous pressures are normal   In other words, there is an abnormally great drop in the pressure gradient between the arterioles and capillaries   Notwithstanding the high pressure in the arteries, the average cardiac output per minute, the stroke volume of the heart, the velocity of blood flow, and the circulating blood volume are slightly less than in healthy individuals

The resistance, which has been demonstrated to exist in the arteriolar portion of the vascular circuit, offers a considerable impediment to the cardiac function and markedly increases cardiac work. The development of the high pressure in the arteries may well be a compensatory response on the part of the circulation to reestablish the normal capillary blood flow and nutrition of the tissues

The basal metabolism averages above the standard for normal persons   The efficiency of exchange of oxygen and carbon dioxide between tissues and capillary blood, judged from the ratio of tissue consumption of oxygen and the corresponding volume of blood flow, is better than in healthy people

This study indicates that in order to gain beneficial results by means of a reduction of high blood pressure, the arteriolar resistance must be diminished   Therapeutic measures which induce a lowering of the blood pressure by lessening cardiac output or decreasing blood volume cannot be of permanent benefit

Cardiotachometric Studies of the Neurogenic Regulation of Ventricular Rate in Auricular Fibrillation   By ERNST P BOAS, New York, N Y

The ventricular rate of patients with auricular fibrillation has been studied by means of the cardiotachometer   It has been shown that the ventricular rate is very variable, that it accelerates in response to the slightest exertion or emotion, and that it slows during rest and particularly during sleep   All of the evidence indicates that the ventricular rate in these patients is under control of the cardiac nerves, and that alterations in rate are governed by neurogenically determined changes in conductivity of the specific conducting tissue of the heart. The changes in ventricular rate arise, apparently, in response to the varying physiologic needs of the body, just as in health, but the reaction is not so well regulated and is often excessive.

Patients with auricular fibrillation may be classed in two groups—those with labile and with stable ventricular rates   The former are high strung and nervous and correspond to patients with neurocirculatory asthenia.   Their ventricular rates tend to be rapid, and quantities of digitalis in excess of the body weight dose are required to keep the ventricular action slow and stable   In addition it would seem that sedative and psychotherapeutic treatment would assist materially in slowing the ventricles   In the stable group the ventricles do not exhibit such an exaggerated response to physical and emotional stimuli, and can be readily kept under control by the usual methods of digitalis therapy

The value of rest and sleep in the treatment of patients with auricular fibrillation, a fact well known, is forcefully demonstrated by actual count of the number of heart beats by means of the cardiotachometer

Observations upon the Refractory Period of the Normally Beating Mammalian Heart   By E COWLES ANDRUS and EDWARD P CARTER, Baltimore, Md

Previous determinations of the refractory period of the mammalian auricle have involved driving the heart with a series of break induction shocks at a rate neces-

sarily more rapid than that of the spontaneous rhythm   The local stimulating effect of these repeated shocks has made accurate measurements possible only upon the atropinized heart, and in many instances, has led to false responses

Using a specially designed apparatus the authors have determined the refractory period of the normally beating dog's heart   The action current is led off from the auricle, amplified to 12 to 20 volts, and applied to a light relay   Activation of the relay releases a pendulum which in turn throws in a single break induction shock at an interval controlled by the position of a tumble switch along its arc   By this means the normal excitatory process is utilized to control the timing of the interrupting shock   In contrast to earlier measurements the results of the authors show a sharp point above which each stimulus produces a response and below which excitation fails to occur

A second difficulty in refractory period determinations in the past has been the necessity of introducing the interrupting shock at some distance from the recording electrodes on account of danger to the galvanometer string   This has been overcome by the use of a balanced circuit with a double induction coil as the source of stimulus and by grounding the proximal lead   With such a circuit it has been possible to place the stimulating electrodes actually astride the proximal lead

A series of measurements has been made under a variety of conditions   The refractory period of the normal dog's auricle has been found to be between 0 08 and 0 12 second   Stimulation of the vagus brings about conspicuous shortening to 0 05 second or even less   A single dose of atropine sufficient to paralyze the vagus causes the refractory period to lengthen   Slow intravenous infusion of adrenalin produces considerable shortening but not to the degree noted under vagal stimulation   Moreover these changes in refractory period are not proportional to alterations in rate, in the case of vagus stimulation and paralysis, rate and refractory period vary in opposite directions

It has been repeatedly observed that a stimulus introduced shortly after the end of the refractory period, during vagal stimulation, is followed not by a single response, but by auricular fibrillation   Such results have been reported by de Boer and others but always in hearts deprived of their blood supply or poisoned with various drugs and never under vagus stimulation alone   This suggests two points of interest (1) Focus of stimulation—in every case the stimulus was applied well out upon the auricular appendix   (2) This rhythm followed stimuli which, in the absence of vagus stimulation, would have fallen within the refractory period   Due to shortening of the refractory period by the vagus they fell upon tissue which was excitable but in which recovery of conductivity had presumably not yet returned to normal   Hence as long as vagal stimulation was continued there existed in the auricular musculature, the conditions favorable to a re-entrant rhythm   The fact that under conditions of increased vagus tone, an extrasystole occurring early in diastole may lead to a re-entrant rhythm is, it seems, significant as a possible explanation of the genesis of auricular fibrillation

Orthopnea. Its Relation to the Increased Venous Pressure of Myocardial Failure
     By A CARLTON ERNSTENE (by invitation) and HERRMAN L. BLUMGART,
     Boston, Mass

The factors, which impel patients with myocardial failure of the congestive type to sit up in bed, have aroused the curiosity of many investigators   Insufficient attention, however has been paid to the rôle of increased venous pressure in causing this phenomenon   The mechanism producing orthopnea in a patient with increased venous pressure due to uncomplicated congestive failure we believe to be as follows   Increased pressure within a vein causes slowing of blood flow in the corresponding capillary tributaries   If a patient with cardiovascular failure and a right auricular pressure equivalent to 15 cm. of water should lie flat in bed, the veins about the respiratory center would have a corresponding pressure of 15 cm   and the blood flow would be diminished   If, however, he should sit up so that the respiratory center were 15 cm. above the right auricle, the pressure within the veins leading from the respiratory center would be zero   The blood flow in the capillaries feeding these veins would then be increased, and the respiratory center might be expected to receive a more adequate blood supply   This should lessen subjective respiratory distress

To test the validity of this theory, 82 comparisons of the height of venous pressure and the degree of orthopnea have been made in 21 patients with uncomplicated myocardial failure   The venous pressure was measured by the direct venepuncture method of Moritz and Tabora, and the degree of orthopnea was estimated by measuring the perpendicular distance between the right auricle and a point corresponding to the respiratory center the external occipital protuberance.   A parallelism between these measurements was, in general observed   the higher the venous pressure, the greater the orthopnea.

Moreover, with the patient flat in bed, simple elevation of the head by flexing it on the thorax conspicuously diminished the respiratory distress in practically every instance   No other theory of orthopnea accounts for this phenomenon

These and other observations indicate that, while other mechanical factors are not without importance, in uncomplicated myocardial failure the orthopneic position reduces subjective discomfort by releasing the respiratory center from the effects of increased venous pressure   This mechanism we believe to be of fundamental importance in the pathogenesis of orthopnea.

Experimental Edema   By LOUIS LEITER (by invitation) and FRANKLIN C Mc
     LEAN, Chicago, Ill

The well known association of low plasma proteins with certain forms of clinical edema and the concept of Starling that the osmotic pressure of the plasma proteins determines the reabsorption of tissue fluids into the capillaries, suggested the production of experimental chronic hypoproteinemia

Dogs were bled 400 to 500 cc. twice daily from the heart and a corresponding volume of erythrocytes suspended in an alkaline Locke's solution was reinjected

intravenously   Fifteen hundred cubic centimeters of 0 85 sodium chloride solution were given daily by stomach tube   The concentration of plasma proteins was determined by the usual micro-Kjeldahl methods

Definite subcutaneous edema with gain in weight appeared by the fifth day in all of the dogs whose plasma protein level fell to about 3 per cent or less   Ascites usually occurred at about the same time   The edema ordinarily involved the external genitals, thighs, buttocks, perineum, and lower abdomen   Discontinuance of bleeding for twenty-four hours led to prompt rise of the plasma proteins and rapid disappearance of edema in spite of continued administration of saline The entire cycle could be repeated on further plasmaphoresis

The subcutaneous edema and ascitic fluids resembled nephrotic edema fluid in appearance, with extremely low protein content—usually less than 0 1 per cent in the former and about 0 25 per cent in the latter   This composition definitely ruled out the increased capillary permeability which is the cause of other types of experimental edema

Control experiments were made to rule out cardiac damage, under-nutrition, and high salt and water intake *per se,* all with negative results   Plasma protein depletion alone does not lead to edema, hence, as in clinical edemas, the salt intake is an important factor   This experimental edema was apparently on an extrarenal and mechanical basis analogous to that seen in some forms of Bright's disease and in undernutrition (war edema and cachectic edema)

The Effect of Edema on Oxygen Utilization   By T R HARRISON and (by invitation) COBB PILCHER, Nashville, Tenn

The oxygen content of arterial blood and of blood from the femoral veins has been studied in patients with heart disease and in individuals without circulatory disorders   The utilization (arteriovenous difference) is less in patients with cardiac edema than in the control group   Average values for numerous analyses were

Arterio-venous difference in volumes per cent

Cardiac patients				Normal controls	Non-cardiac edema
Marked edema	Moderate edema	Slight edema	No edema		
4 08	5 15	5 44	6 52	7 73	4 22

There were two exceptions to these general findings   In patients with (*a*) digitalis intoxication or (*b*) acidosis, the utilization was high, whether edema was present or not

The low utilization in patients with edema was noted whether they were gaining or losing edema   These observations are interpreted as follows   Since there is hyperoxemia of the femoral venous blood in patients with edema of the legs, increased blood flow through the edematous legs seems probable   This may be due to local oxygen lack in the tissues if, as seems likely, edema in the tissues, as in the

lungs, causes a partial obstruction to the passage of oxygen through the capillary wall. Hence, the oxygen pressure may be raised in the blood and lowered in the tissues. It seems that edema *per se* throws an additional strain on the heart by causing additional circulation to the edematous tissues and hence, that a vicious cycle may ensue   Therefore it is probable that the cardiac patient suffers not so much from cardiac insufficiency as from circulatory inefficiency

It should be emphasized that the results found seem to be primarily effects, and only secondarily causes of edema

A Study of Serum Inorganic Sulfates in Renal Insufficiency   By E G WAKEFIELD
      (by invitation) and N M KEITH, Rochester, Minn.

Further Studies on Deposit Nitrogen   By G P GRABFIELD, Boston, Mass.

In the attempt to apply previous studies on the mobilization and excretion of nitrogen in the body by means of iodides and salicylates to patients with chronic Bright's disease, it was found that patients showing the nephrosis syndrome excreted large amounts of nitrogen in proportion to the sulphur excretion   In comparing cases of Bright's disease without edema with those presenting the nephrosis syndrome, it was found that the former excreted urine, presenting the normal nitrogen-sulphur ratio and that these patients tended to exhibit negative sulphur balance   Patients presenting the nephrosis syndrome, on the contrary, showed a strong tendency to retain sulphur as compared with their retention of nitrogen   Such patients retained as much as 85 per cent of the sulphur of the diet.   In a general way, of the patients presenting the nephrosis syndrome those that retained on high protein diets most sulphur in relation to the retention of nitrogen, showed the best clinical results in loss of edema and sense of well being

These patients were kept for long periods on diets constant as to nitrogen, sulphur and phosphorus.   The water intake was controlled and kept at a definite level by calculating the water content of the food and adding sufficient fluid to the diet to make a constant volume.

It was found that many of these cases were mixed and that some approached on lower protein diets the ideal condition in relation to nitrogen and sulphur retained

The basis for further study of the sulphur metabolism in Bright's disease has been laid by these experiments, and it seems not unlikely that this component of the protein molecule will prove to be a factor of greater importance than it has hitherto been considered to be

Liver Fractions in Pernicious Anemia   By RANDOLPH WEST, and (by invitation)
      MARION HOWE, New York, N Y

We have shown that the substance in liver effective in pernicious anemia is precipitable by phosphotungstic acid but not by silver even in alkaline solution. The process now used is as follows

Four hundred grams of Lilly Liver Extract is dissolved in 600 cc water and to this 200 grams anhydrous Na_2SO_4 are added   After filtering off the precipitate which forms, sufficient 95 per cent alcohol is added to the filtrate to give a final concentration of 60 per cent   The supernatant alcoholic fluid is poured off within a few minutes after adding the alcohol and filtered   Alcohol is blown off from the filtrate with a hair dryer till volume is about 500 cc   The Kossel silver fractionation is then carried out using silver lactate and baryta, and excess silver removed from the filtrate as sulphide or chloride and baryta as sulphate   To the silver filtrate 3 per cent by volume H_2SO_4 is added, and then a moderate excess of phosphotungstic acid (Kahlbaum)   The phosphotungstic precipitate is dissolved in 75 per cent acetone and decomposed with hot aqueous baryta and filtered at the pump   Excess baryta is removed as sulphate, acetone blown off, and the material is ready for feeding   Yield about 3 to 4 grams solids   This fraction has never failed to give positive results on feeding in pernicious anemia   The best preparation fed to a case of tropical sprue in doses of 0 5 gram daily for eight days gave a reticulocyte peak of 200,000 per cubic millimeter, red blood cells being about 1 9 million   The poorest preparation fed in doses of 1 5 gram daily to a case of pernicious anemia gave a reticulocyte peak of 360,000 per cubic millimeter with red blood cells at 1 2 million   This is known as fraction B   It gives positive biuret, indole and diazo reactions, also a positive "arginine" reaction with a naphthol and sodium hypochlorite

On treating fraction "B" with mercuric acetate at pH 6 0 practically all material precipitable with phosphotungstic acid is removed from the solution, but on regeneration of the precipitated bases with H_2S and feeding in 0 6 gram doses daily we have had negative resutls (one case tropical sprue with prompt response later) to 12 grams Lilly Extract daily

On adjusting fraction B to pH 7 0, and adjusting volume so that the material derived from 800 grams Lilly Extract was in 100 cc water, and pouring this into 900 cc of acetone, the biuret positive material was largely precipitated   A case of pernicious anemia was given 1 5 gram of this precipitate, derived from several kilograms of Lilly Extract, daily for eight days with a negative result, and later responded well to liver   The acetone soluble material, after blowing off acetone, gave a strong diazo and indole reaction and an extremely faint biuret   This in doses of 0 6 and 0 8 gram daily has given weak positives (reticulocyte peaks of about 140,000 per cubic millimeters with red blood cells below 2 0 millions) in two cases, and a negative in one case

On evaporating fraction B to small volume and making up to 100 cc (material from 400 grams Lilly) with absolute alcohol to give final alcoholic concentration of 74 per cent and treating with an excess of alcoholic platinic chloride a precipitate formed which on regeneration with H_2S and reprecipitation with platinic chloride was crystalline   The platinum content and the melting point of the aurichloride and mercurichloride agree with those of choline   Mixed melting point with known choline aurichloride showed no depression   An insoluble iodide,

resembling choline iodide in crystal form was also observed. On feeding this base in doses of about 700 mgm. daily for ten days to a case of pernicious anemia with red blood cells at one million per cubic millimeter the reticulocytes rose from 4.2 to 11 4 per cent. This material had been through platinum but once, and was derived from 4 kgm. of Lilly Extract. The effect was probably due to impurities accompanying choline A second case fed 120 mgm daily of material, twice through platinum, showed on the eleventh day of feeding a rise of reticulocytes, from 1 to 7 per cent with red blood cells at 1 0 million

The platinum mother liquor, after removing Pt with H₂S on feeding 5 5 grams in one dose gave a reticulocyte peak of 150,000 per cubic millimeter with red blood cells at 1 7 million.

Synthetic choline, on feeding six grams in three days gave no reticulocyte re sponse on the seven days following, whereas feeding 72 grams Lilly Extract in two days gave a reticulocyte peak of 300,000 per cubic millimeter with red blood cells at 1.2 million on the seventh day after feeding

Fraction B has retained its full activity after removing all material precipitable with picric acid (4 grams solids in 20 cc. solution saturated with solid picric acid) and after shaking with butyl alcohol in both acid and alkaline solution

Serum Iron Studies By HERMAN H RIECKER and MARY E WINTERS (by invi tation) and HENRY FIELD, JR., Ann Arbor, Mich.

The object of this study was to determine whether or not a relationship existed between (1) the level of serum iron, hemoglobin production, and iron administra tion in the experimental anemia of hemorrhage, (2) the level of serum iron and the iron metabolism in pernicious anemia during active remission, and (3) the serum iron content and the hemoglobin percentage in various secondary anemias

Iron determinations were made by Elvejhem s and Hart s modification of the Thompson method. The element of error possible from hemolysis was avoided by precipitation of the serum proteins with trichloracetic acid, following the sug gestion of Briggs

The animal experiments were conducted upon dogs by a method essentially similar to that devised by Whipple and Robscheit Robbins

The following relations were noted As the anemia progressed there occurred a depression in the serum iron level from the normal of about 1 2 mgm. per 100 cc. of blood to between 0 7 and 0.9 mgm during which time the animal did not re generate hemoglobin When Fe was added to the diet in any form regeneration began and the serum iron level definitely rose

Then a series of six pernicious anemia cases were studied during relapse and re mission Blood serum iron determinations were made and the intake and excre tion of iron were determined The serum iron figures were all above, or within, the normal limits

The iron excretion of patients before treatment on a constant intake of 7 to 15 mgm. was found to be fairly constant, as was the control case. However, when

treatment was begun with liver extract a marked increase of excretion over intake occurred and continued throughout the periods of observation

In the secondary anemias those due to hemorrhage were separated from those of an unidentified nature   In cases with acute hemorrhage the serum iron levels were in general lower than in the other types   The studies in secondary anemia have not been completed so that the results given merely indicate the trend of the investigation

Conclusions   1   In the experimental anemia of hemorrhage a definite iron starvation is present and this is relieved by iron feedings with a rapid increase in hemoglobin production   Serum iron determination may be used to detect this condition

2   In pernicious anemia there is a well marked negative iron balance during the remission induced by liver extract   The serum iron levels tend to be increased above the average normal in relapse, and to approach the normal in remission

3   No degree of iron stravation could be found in secondary anemias comparable to that in experimental animals with anemia due to hemorrhage

The Endogenous Uric Acid Metabolism in Pernicious Anemia   MATTHEW C RIDDLE, (by invitation) and CYRUS C STURGIS, Ann Arbor, Mich

The endogenous uric acid metabolism was studied during early remission in twelve patients with pernicious anemia, using purine-poor diets over periods of 10 to 75 days

Before treatment, in two patients the concentration of uric acid in the blood serum was higher than normal, in three it was normal, and in six lower than normal   Only one patient excreted increased amounts of uric acid before treatment

During remissions induced by liver extract therapy there was an increase in the urinary excretion of uric acid of from 74 to 531 per cent and an increase in concentration of uric acid in the blood serum of from 28 to 239 per cent which was correlated with the rise in percentage of reticulocytes   The increased uric acid metabolism was shown to be endogenous in nature by the failure of impotent liver extract to produce the typical results, by the typical uric acid increase both following the administration of a single massive dose of liver extract and during spontaneous remission and by the failure of liver extract to increase the uric acid metabolism in a normal person

The increased uric acid metabolism may be associated with the increased rate of destruction of red blood cell nuclei and a general increase in nuclear metabolism during remission

Calcium Metabolism in Hyper- and Hypo-Parathyroidism   By H A BULGER and D P BARR, St Louis, Mo

Recent advances in our knowledge of calcium metabolism, especially those afforded by Collip's parathyroid extract, have placed us in a position to recognize the clinical picture of hyperparathyroidism   Extensive study of two such patients

has revealed typical symptoms and associations, together with characteristic abnormalities of calcium metabolism More calcium than ingested was excreted by the intestines alone, although there were also excessive amounts in the urine. There was a negative phosphorus balance

A great reduction in calcium in the urine and a positive calcium balance could be obtained by administration of phosphates

These abnormalities were compared with and were in striking contrast to those found in hypoparathyroidism, in which there have been almost negligible amounts of calcium in the urine and a remarkable tendency to retain calcium

One patient with hyperparathyroidism was studied in all stages of transition to extreme hypoparathyroidism which followed the removal of a parathyroid tumor

The Specific Dynamic Action of Protein in the Obese By E F Du Bois and (by invitation) H. J Spencer, W S McClellan and E Falk, New York, N Y

One explanation of obesity is that the specific dynamic action of food stuffs is below normal. There is considerable doubt, judging from the literature, whether this is a fact We have studied the influence of protein in obese and normal individuals by means of the respiration calorimeter

Twelve observations following meals of 300 to 500 grams of lean meat were made on 4 obese and 3 normal men of various ages who were studied for considerable periods in the metabolism ward These observations show considerable variation in the heat output from hour to hour both in the individual records and in the group averages The extra heat production, expressed as percentages of the basal heat out put has been calculated hourly from the time the meat was ingested

The average increase in caloric output of the obese men surpassed the normal in the fourth hour The normal men put out heat more uniformly The average surplus heat output per hour was approximately 13 per cent for the obese, and 17 per cent for the normal men

Our impression is that, while the obese man responds to protein more irregularly than the normal man, his extra heat output is not significantly different from the normal

The Effect of Lessened Respiratory Reserve on the Blood and the Circulation An Experimental Study By Willis S Lemon, Rochester, Min

A series of experiments was carried out with the object of observing the effect of lessened mechanical efficiency on respiratory reserve It was hoped also to discover the point at which reserve is lost, respiration as a function becomes inadequate and circulatory aid is required to maintain comfortable existence The animal studied was an intelligent dog which had previously been taught to lie quietly while wearing the mask usually employed for basal metabolic estimations

At approximately weekly intervals during March and April of 1927, the dog was subjected to four operations carried out under ether anesthesia and aseptic tech

nic   These operations were (1) evulsion of all the left intercostal nerves, (2) evulsion of the lower eight right intercostal nerves, (3) evulsion of the right phrenic nerve, and (4) evulsion of the left phrenic nerve

As a result of this procedure, respiration depended on the actions of the muscles attached to the ribs posterior to the operative sites, and those extra-respiratory muscles about the neck and shoulders which are used normally only following extreme muscular exertion   The respiratory function of the diaphragm was of course excluded, and its effect on intraabdominal pressure removed, its only rôle was that of a partition separating thorax from abdomen

The behavior of the dog following surgical interference was in every respect similar to that observed in a previously reported group of experiments   As a result of section of the intercostal nerves on one side, there was immediate or delayed, temporary or persistent, asymmetry of the chest, with a definite and measurable loss in outward movement of the costal arch and margin during inspiration   The reduction amounts to from 30 to 50 per cent of normal, depending on the number of nerves cut   When half the diaphragm is paralyzed, no outward movement of the costal arch margin occurs during inspiration   Symmetric reduction of movement results from bilateral section of the intercostal nerves, and regardless of the order in which phrenic nerve paralysis is introduced, the movements remain the same   The animal of the present experiment after operation lived comfortably, played about like its normal mates and on casual examination could not be distinguished from them   It could not, however, respond to vigorous exercise with the same ease owing to the limitation of vital capacity

In accordance with the object of this investigation, the intake of air for each unit of time was measured both before and after the series of operations, since it was believed the results would be equivalent in value to alterations in volume   The figures indicated that the respiratory function was adequate to supply sufficient ventilation under all conditions and that the vital capacity provided for a normal amount of tidal air

The animal was returned to the kennels and apparently lived a normal life during the following year   Despite the paralysis of the whole diaphragm and upper abdominal muscles, difficulty in urination or in defecation was not observed   The lower abdominal muscles, however, had undergone hypertrophy

Exactly one year after the last operation, the abdomen was opened   The diaphragm was found to be high in the thorax and so thinned that the lungs were plainly visible through it   Specimens from each leaf of the diaphragm were removed for study   After the animal had recovered from the operation, it was observed that as the chest was raised during inspiration, the lower abdominal muscles contracted strongly   This suggests a compensatory attempt to retain the abdominal contents in the abdomen, thus preventing respiratory embarassment

Twenty months after the first operation, the blood was studied to determine whether or not the long continued reduction in vital capacity could have brought

about erythrocytosis, changes in blood volume, in hematocrit values, in hemoglobin, in oxygen, in carbon dioxide, or in the blood count. Electrocardiographic tracings were also made   Repeated calculations and comparisons with values obtained in normal dogs did not reveal significant alterations in the experimental animal. This confirms the opinion that the animal retained sufficient respiratory reserve and under ordinary living conditions did not require assistance from the circula tion

At the end of February, 1929, a second laparotomy was performed.   After the adhesions were separated, it was found that the stomach, part of the duodenum and all but two of the lobes of the liver had migrated up into the thorax, this confirmed previous roentgen ray data   The part of the diaphragm which yet remained was, as previously described, thin and translucent and flapped back and forth with respiratory movements   An incision through its costal attachments was made on each side thus eliminating its action as even a partition   The abdo men was then closed and on recovery from the anesthetic the dog ran about the room without any apparent respiratory embarrassment.   A regimen of rest was imposed to encourage healing and particularly to allow development of compensa tion and to avoid unnecessary strain

Examinations of the blood, March 8 and 16, showed, respectively, hemoglobin 85 and 90 per cent, erythrocytes 4,910,000 and 5,760,000 and leukocytes 15,900 and 12,400 for each cubic millimeter   The differential count was essentially nor mal as also was the percentage volume of erythrocytes.   The air intake each minute was somewhat larger than previously, being 4.56 liters as compared with 3 18 preoperatively   The respiratory rate each minute had increased to 22 from a preoperative average of 12   The pulse rate remained the same   Hematocrit varied from 42 to 45 per cent of cells   Blood volume was 86 cc per kilogram The electrocardiogram was normal

Summary   Vital capacity has been lessened, but never beyond being equivalent to tidal air, ventilation volume was raised somewhat, but never to an abnormal degree.   The hemoglobin remained within the normal limits as also did the hema tocrit percentages   Although the oxygen saturation of both arterial and venous blood was lowered the arterial and venous oxygen content was essentially normal There was no erythrocytosis and the heart showed no evidence of abnormality

These findings would seem to confirm the independence of the fundamental functions of the cardiac and respiratory systems, great loss of reserve in one ,being necessary before demonstrable evidence of coöperative support is provided by the other

The Experimental Production of Chronic Abscess of the Lung   By Louis G Herr mann, (by invitation) and Elliott C Cutler, Cleveland, Ohio

A study of the factors that are responsible for the chronicity of the suppurative processes of the lung was made.   The marked increase in the intrabronchial pressure associated with constant violent cough has been shown to play a minor

rôle in the prolongation of the activity of these pulmonary lesions   In the experiments previously reported from this laboratory only aerobic microorganisms were implanted in the pulmonary tissue by means of the vein-segment embolus   The lesions that resulted were all of the acute type and they healed spontaneously within three weeks

Recently Smith showed that the bacteria present in chronic abscess of the lung in human beings were morphologically identical with the bacteria present in the material from the margins of the gums of patients with pyorrhoea alveolaris

A detailed study of this material was made and the embolic method of implantation of this material into the pulmonary tissue was utilized in a series of 38 dogs   With one exception each animal developed a localized abscess at the site of lodgment of the embolus   Of the 38 dogs 28 9 per cent developed pulmonary abscesses which remained active for more than five weeks   In two of the animals the lesions remained active for 101 days and 126 days respectively   At autopsy the cavities of the abscesses were filled with grayish-green necrotic material that had a very foul odor   There was a thick wall of induration about the cavity of the abscesses

The material from these experimentally produced chronic pulmonary lesions was cultured on a great variety of culture media and under both aerobic and anaerobic conditions   In all, twelve different varieties of microorganisms were isolated in pure culture,—five aerobic microorganisms and seven anaerobic microorganisms

These pure cultures of microorganisms were then implanted into the pulmonary tissue of another series of dogs   The aerobic organisms produced only acute lesions that healed spontaneously within three weeks   The lesions that resulted from the implantation of the anaerobic microorganisms in the pulmonary tissue were of longer duration and several of them remained active for more than five weeks   Pure cultures of the *Spirochaeta microdentium* and the large and small variety of fusiform bacilli were also implanted in the pulmonary tissue but no lesion developed at the site of lodgment of the embolus

Several combinations of these anaerobic microorganisms were used and the lesions that were produced more closely resembled the true chronic abscesses of the lung which are found in man

Our studies show that the anaerobic group of organisms are the important factors that determine the chronicity of pulmonary lesions   Further experiments are being conducted with the hope of determining the exact combination of anaerobic microorganisms that is responsible for the chronicity of these lesions

The Flora of the Upper Respiratory Tract of Infants in the First Year of Life   By
     YALE KNEELAND, JR , (by invitation) and A R DOCHEZ, New York, N Y

In connection with studies on the common cold, it was thought advisable to investigate the upper respiratory flora of infants   This report is based on data derived from thirty normally-delivered infants in Sloane Hospital, a number of

these were subsequently followed in the out-patient department, and observations were also made on infants of the same age with and without colds As a rule the upper respiratory tract is sterile at birth, no growth being obtained in 85 per cent of the nasal cultures, and 80 per cent of the throats in the first day of life After the first feeding however, bacteria are regularly found in the throat, although the nose may remain sterile for another 24 hours Growth from the nose is rarely abundant the *Staphylococcus albus* being the most prominent organism. Diph theroid bacilli rank next, with *Staphylococcus aureus* and large Gram positive cocci following Streptococci are relatively less frequent, occurring in but 19 per cent of all cultures Members of the colon group and Gram negative cocci are rarely encountered From the throat growth is usually abundant with streptococci the outstanding organism, the indifferent (gamma) type being more frequent than the green producing varieties Staphylococci are also very common Colon bacilli are occasionally seen, while diphtheroid bacilli and Gram negative cocci are rare In the first two weeks of life, *B pfeiffer*, pneumococcus and hemolytic streptococ cus were never encountered either in the nose or throat.

For comparison with this group, twenty eight normal infants from four to eight months of age were studied, and certain differences noted In the nose the staphy lococci were less outstanding, with a corresponding rise in the Gram negative cocci (18 per cent as opposed to 1 per cent in the new born), and the appearance of *B pfeiffer* and pneumococci in 7 and 14 per cent of the cases respectively The throat showed a marked increase in the green streptococci, which appeared in 100 per cent of cases, at the expense of indifferent streptococci and staphylococci The incidence of Gram negative cocci went from 4 per cent to 71 per cent, and *B pfeiffer* was found in 53 per cent of cases. Hemolytic streptococci appeared in 11 per cent (Nineteen of these normal infants gave a history of having had colds and nine did not, there was no difference between the two save for a somewhat higher incidence of Gram negative cocci in the throats of the former)

Observations were also made on twenty-one infants early in the course of their first colds, the average age being three months There was no essential differ ence between their flora and that of the normal group, with the exception that they showed a higher incidence of diphtheroid bacilli, and pfeiffer less frequently From a clinical standpoint, these first colds are mild, characterized by a moderate degree of coughing and sneezing, with at times a little coryza, usually without constitu tional symptoms

Lastly, a group of fourteen infants with recurrent colds were studied, the average age being seven months. One striking change was noted from the normal con trols, namely the increase of *B pfeiffer* and pneumococci in the nasal cultures to 21 and 43 per cent respectively The latter was actually predominant in 36 per cent of cases It may be added that many of these children had a purulent nasal discharge, and sinusitis was not improbable The flora of the throat remained unchanged

From the evidence thus far obtained, it would appear unlikely that there is any

specific bacterial incitant of the early colds of infancy, although in the chronic recurrent type of cold bacteria probably play a part   On the other hand, such pathogens as *B pfeiffen*, pneumococcus, and hemolytic streptococcus may occur in normal infants without giving rise to symptoms

Chloride Balance in Pneumonia[1]   By J H Austin and (by invitation) F W Sunderman, Philadelphia, Pa

The intake and output of chloride have been measured in seven patients with lobar pneumonia   Four of these patients received the usual diet and therapy, three received large amounts of sodium chloride (8 to 30 grams per day) by mouth during the precritical period   In the first group although during the precritical period excretion of chloride in the urine was very low nevertheless the total excretion of chloride exceeded the intake   In the second group the excretion of chloride in the urine was increased but was less than in normal subjects   In the second group there was consistently retention of chloride before the crisis   Our studies support the view that lobar pneumonia is characterized during the precritical period by a diminished capacity to conserve chloride on a low intake of chloride and a diminished capacity to excrete chloride on a high intake of chloride

The Experimental Reproduction of the Blood Picture of Infectious Mononucleosis in the Guinea Pig   By L W Gorham and (by invitation) David T Smith and H D Hunt, Albany, N Y

Infectious mononcleosis is a benign sporadic disease occurring in young adults, and is characterized by fever, sore throat, and enlargement of the glands and spleen   Although Turck studied a case in 1907 the real interest in this disease dates from the contribution of Sprunt and Evans in 1920

The frequent occurrence of sore throat in these cases suggested that the infectious agent either remained localized to the pharynx or entered the body through the pharynx   Some of the cases present typical Vincent's angina of the pharynx, while others do not

The first case studied was a typical case of infectious mononucleosis in a young girl who had a very severe Vincent's angina of the tonsils   Some of the membrane from the pharynx was inoculated into the groin of two guinea pigs and both animals developed the typical blood picture of infectious mononucleosis   Dr H. D Hunt developed infectious mononucleosis subsequent to his contacts with the patient and with the infected guinea pigs   The mother of Dr Hunt also developed the disease apparently as the result of direct contact   Material removed from the throat of Dr Hunt with a suction apparatus produced the blood picture of the disease in the guinea pigs   Later in the experiments Miss R , who was counting the blood of the infected guinea pig, developed infectious mononucleosis   Material removed from her throat also produced the typical blood picture in

guinea pigs So the blood picture of the disease has been reproduced in guinea pigs with material from the pharynx of three different cases One of these had a typical Vincent's infection of the throat, one had a typical Vincent's infection but the throat did not have Vincent's infection.

An examination of Dr H D Hunt's throat in the early stage of the disease revealed a very large number of vibrios Since very little is known of the blood reaction to vibrios we decided to isolate and study this organism first. Two strains were isolated, One from a case of Vincent's angina and one from a case of acute Vincent's infection of the bronchi Both these strains produced typical blood pictures in the guinea pig One of these strains was used only as a dead heat killed vaccine This dead material produced just as typical changes in the blood as did the living organism of the other strain After recovering from one attack the animals were immune to a second or third inoculation There was cross immunity between the two strains Blood from Dr Hunt agglutinated a vibrio from a different source in a dilution of 1 64 Two cubic centimeters of this serum prevented the appearance of the blood changes in the guinea pig for one week, after which time the typical changes appeared A rabbit was immunized to the vibrio and its blood agglutinated the organism in a dilution of 1 320 Five cubic centimeters of this serum prevented the development of the blood changes in a guinea pig inoculated with 2 cc. of a vibrio culture Five cubic centimeters of blood from a normal rabbit failed to protect a guinea pig inoculated with the same amount of same culture.

Cultures of (1) fusiform bacilli, (2) Streptothrix (3) *Staphylococcus albus* (4) *Staphylococcus annus* (5) *Streptococcus hemolyticus* (6) *Streptococcus viridam* (7) *Bacillus coli* and (8) diphtheroid all failed to produce this typical picture.

The Effects of Over Dosage with Irradiated Ergosterol By A T Shohl and (by invitation) Harry Goldblatt, Cleveland, Ohio

Rats were fed normal diets and also ricketogenic diets of both the high calcium and low phosphorus type and high phosphorus and low calcium type Four mgm. of irradiated ergosterol (Vigantol) were administered daily They died approximately in seven days Marked calcification of the blood vessels of the heart, kidneys and stomach was observed and hypercalcification of the epiphyses of the long bones Calcium and phosphorus of the blood serum and the ash of the fat free bones were determined. Control experiments with unirradiated ergosterol or olive oil showed none of these abnormalities

Studies on the Value of Acid Neutralization in the Treatment of Gastroduodenal Ulcer By Joseph A. Capps and (by invitation) Walter Lincoln Palmer, Chicago, Ill

In this paper a comparison is made between two similar groups of patients, each comprising 27 cases of gastric and duodenal ulcer treated under the same conditions as regards rest and gastric evacuations, with the same diet and with

hourly feedings of milk and cream   Alkaline powders dissolved in 90 cc of water were administered hourly in one group in doses large enough to neutralize the gastric free acidity as completely as possible throughout the entire digestive period, in the other group, 90 cc of 10 per cent Liebig's beef extract was given every hour in place of the powders, the idea being to stimulate gastric secretion thereby The duration of spontaneous pain was observed in each group and every fourth day an "acid test" performed for the purpose of ascertaining the duration of sensitivity to this test   The results are based upon this comparison   The alkali group showed a much more prompt cessation of spontaneous pain than did the beef tea group and became acid insensitive more quickly

A Study of the Reflex Influence of the Colon on the Stomach   By FRED M SMITH, and (by invitation) GEORGE H MILLER, Iowa City, Iowa.

There is a considerable difference of opinion relative to the possible reflex influence of the colon on the stomach   Epigastric pain, nausea, and vomiting are frequently associated with chronic colitis   Clinicians and roentgenologists have been inclined to attribute these symptoms to a disturbed motor function of the stomach induced by a reflex stimulation from the colon   The experimental investigation of this problem, however, has led to varying conclusions

In the present investigation, dogs, anaesthesized by barbital were employed A mid line incision was made in the upper abdomen which would permit exposure of the stomach and proximal colon   The stomach was filled with warm water and the activity observed during a control period   Croton oil was then introduced into the proximal colon   Precautions were taken to prevent the irritant coming in contact with the ileum   In some instances the bowel was later distended with air   Following the introduction of the irritant into the colon there was usually a marked increase in the activity of the stomach   The peristaltic waves were much more frequent, deeper, and at times very violent   During the height of the increased activity, reverse waves were occasionally noted   The activity of the stomach was often further intensified by distending the colon   In experiments in which the irritation was confined to the appendix, the effect on the stomach was similar   The activity of the stomach induced by the stimulation of the colon or appendix was abolished by atropin

The Systemic Effect of the Presence of Bile in the Peritoneal Cavity   By G O BROWN and (by invitation) A P BRIGGS, St Louis, Mo

Whole bile or solutions of bile salts introduced into the peritoneal cavity give rise to a severe serofibrinous peritonitis   Vomiting and diarrhea occur leading to dehydration   There is a marked fall in blood pressure and extreme prostration and weakness   The heart rate may be slowed in some cases   In others it becomes more rapid   The electrocardiogram shows some irregular changes in the T-waves   Secretion of urine is decreased or completely suppressed

Changes in blood chemistry are those expected with dehydration and decreased

urinary output Blood chlorides show a moderate fall Non protein nitrogen and inorganic phosphate are markedly increased Plasma protein increased slightly Changes in blood lipoids and blood sugar were irregular and not marked Tests for the presence of bile salts in the blood stream gave positive reactions In dogs no increase in blood bilirubin was found although bilirubinuria occurred.

Concentration of the blood is shown by decided increases in red count, cell hematocrit and hemoglobin content. A marked polymorphonuclear leucocytosis occurs A moderate increase in the fragility of the red corpuscles is observed Some free hemoglobin may be found in the blood plasma. In the absence of infection, erythrocyte sedimentation rate is delayed Clotting time is slightly delayed but may remain within normal limits

The Respiratory Metabolism of the Tubercle Bacillus [1] By R. O. LOEBEL, E. SHORR (by invitation) and H B RICHARDSON, New York, N Y

In the study of tuberculosis it is desirable to know the processes by which the parasite obtains energy and the influence of adverse conditions on its respiratory metabolism

The following is a study of the oxygen consumption of the tubercle bacillus as influenced (1) by the age of the culture (2) by the constituents of the nutrient medium, and (3) by the pH of the solution

After 8 days cultivation on Long's synthetic medium the consumption of oxygen, measured in the micro-respiration apparatus of Warburg, averaged 2 6 cmm. per milligram of moist weight, per hour, after 15 days, 2.2 after 22 days, 0 45, and after 29 days practically zero The maximum was about twice as much as reported for mammalian leucocytes It was about one fifth that of a rapidly growing organism of the acid fast group i e , the smegma bacillus.

In order to establish a base line for the study of nutrients the organisms were transferred from Long's medium to a similar solution containing only the salts of sodium and potassium After 6 days the respiration became much reduced, but on retransfer to Long s medium regained nearly its original level The oxygen consumption in Long s medium could be fractionated as follows

	per cent
Starvation level	6
Effect of glycerol	73
Effect of nitrogenous substances (asparagin and ammonium citrate)	19
Effect of ferric ammonium citrate and magnesium sulfate.	2

After starvation the organisms retained their original power of growth

No increase in respiration above the starvation level was observed as the result of transfer from buffered saline to a similar solution containing 5 per cent glucose

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[1] Aided by a grant from the Research Committee of the National Research Association

or levulose This is in accord with the well known difficulty of cultivating the organisms by the use of sugars Sodium lactate caused a rise which even exceeded the effect of glycerol, but was entirely ineffective as a substitute for glycerol in the promotion of growth This is an instance in which the energy metabolism is dissociated from growth

TABLE 1

The influence of foodstuffs on the growth and respiration of the tubercle bacillus—II 37

Duration	Oxygen consumption per moist milligram					Remarks
	No foodstuff	Glycerol 5 per cent	Na lactate 5 per cent	Glucose 5 per cent	Levulose 5 per cent	
hours	*c mm*	*c mm*	*c mm*	*c mm*	*c mm*	
1	1 1	1 8	2 1	1 3	1 2	16 d culture 2 d starvation
1	0 6	1 5	2 4	0 9	0 5	16 d culture 2 d starvation
1	1 0	1 2	1 6	0 9	0 7	15 d culture
2	1 8	2 4	3 3	1 8	1 5	2 d starvation
	Growth on a modified Long s medium*					
	None	Profuse	None	Slight	None	

* The foodstuff at the head of the column was substituted for the glycerol of Long's medium

In saline solutions buffered with phosphate mixtures the oxygen consumption for short periods was much the same over a range extending from pH 1 5 to 12 0

TABLE 2

The influence of hydrogen ion concentration on respiration of the tubercle bacillus—II 37

Duration	Oxygen consumption per moist milligram					Remarks—phosphate buffers M/150
	pH 1.5	pH 5 6	pH 7 4	pH 8 8	pH 12 0	
hours	*c mm*	*c mm*	*c mm*	*c mm*	*c mm*	
1	1 0	1 0	1 1	1 1	1 2	16 d culture 3 d starvation
1	1 3	1 6	1 6	1 4	1 5	10 d culture
2	2 3	3 3	2 8	2 6	2 8	
3	3 3	5 1	4 4	4 0	4 3	
1		1 7	1 8	1 5		10 d culture

The Chemical Metabolism of Normal and Diseased Lymph Nodes By HENRY
JACKSON JR , and (by invitation) FREDERIC PARKER JR., and E C GLOVER,
Boston, Mass

For years there has been much discussion as to the nature of those diseases
which may be classified under the general heading malignant lymphoma or lym
phoblastoma   Some pathologists hold that they are true malignant processes,
while others maintain that they are infectious or granulomatous in nature   War
burg has shown that with certain exceptions malignant disease tissue has a characteristic metabolism   In brief neoplasms use but little oxygen but consume
large quantities of sugar under both aerobic and anaerobic conditions, whereas
normal organs destroy but little sugar either in the presence or absence of oxygen
and embryonic material destroys but little sugar under aerobic but large amounts
under anaerobic conditions

We have examined the metabolism of twenty two malignant lymphomas and
compared these with the metabolism of normal and tuberculous nodes and with
nodes containing metastatic malignancy   We have also included determinations
on several benign tumors

Our findings may be summarized as follows

Average glycolysis
(Milligrams per milligram hour)

	Aerobic	Anaerobic
Normal nodes	0 020	0 035
Lymphomata	0 050	0 075
Tuberculous	0 075	0 106
Carcinoma	0 110	0 134

Extremes of glycolysis
(Milligrams per milligram hour)

	Aerobic	Anaerobic
Normal nodes	0 00–0 05	0 01–0 075
Lymphomata	0 02–0 10	0 04–0 140
Tuberculosis	0 05–0 13	0 08–0 19
Carcinoma	0 07–0 17	0 09–0 23

In order to overcome certain exceptions and difficulties Warburg introduced a
value ' U' which was the anaerobic glycolysis less twice the oxygen consumption
This value he found to be negative for normal, zero for embryonic and a high posi
tive for cancerous tissues

Calculating this figure from our data we find

Value for "U"

Benign tumors	2 1
Normal nodes	4 3
Lymphomata	10 0
Tuberculosis	10 2
Carcinoma	28 7

It is difficult to draw definite conclusions from these data at the present time More work is being done and the data elaborated   For the present we would point out

1   There is considerable overlapping of the various classifications   By metabolic figures alone the tissue cannot be surely classified   The value U is more consistent

2   From the value U one would place the malignant lymphomata among the granulomatous rather than the neoplastic diseases

3   Further study is being made of the relation of the metabolic changes to the particular cell involved.

The Effect of Iron on Blood Formation as Influenced by Changing the Acidity of the Gastric Contents in Certain Cases of Anemia   By STACY R METTIER, (by invitation) and GEORGE R MINOT, Boston, Mass

The oral administration of iron produces in certain cases of "secondary" anemia a response of the reticulocytes   The anemia in the 4 cases reported upon was associated with defective diets and disorders of digestion or chronic blood loss In these cases when the gastric contents was made neutral in reaction, the feeding of a given daily amount (0 08 to 0 32 gram) of iron produced a response of reticulocytes   Soon afterwards, however, when the same dose of iron was maintained, a second and greater response of these cells occurred after the gastric contents was rendered acid

In the same case two distinct responses of the reticulocytes within a short period of time may be interpreted to indicate that the cause of the second response is more potent than that producing the first   Furthermore, during a third period when the amount of iron was increased four fold or more in these cases, there developed a third response of the reticulocytes   This indicated that iron was the essential factor in producing the response, modified by changes in the acidity of the gastric contents, and is distinct evidence that large doses of iron may be much more effective than relatively small ones   The patients with great rapidity increased to normal the concentration of their hemoglobin and red blood corpuscles

Urobilinogen Excretion in Addison's "Pernicious" Anemia Before and After Liver Therapy   By DUNCAN GRAHAM and (by invitation) RAY FARQUHARSON, HENRY BORSOOK, AND A M GOULDING, Toronto

In Addison's "pernicious" anemia in state of relapse with anemia the urobilino-

gen excretion is greatly increased and may be several times the normal value This is usually associated with a high bilirubin content of the blood plasma Following liver therapy and beginning about the peak of the reticulocyte response there is always a sharp decrease in urobilinogen excretion which falls in a few days to a point within normal limits In severe cases the amount of urobilinogen excreted per day is equivalent to 9 to 12 grams of hemoglobin and in one case it amounted to one-eighth of the total blood hemoglobin of the body

In some cases of Addison's "pernicious" anemia showing little anemia but presenting other manifestation of the disease such as subacute combined degeneration of the cord, the blood bilirubin is usually normal but the urobilinogen excretion is increased somewhat above a high normal value. On liver therapy such cases show a low reticulocyte response and the urobilinogen excretion falls slowly and gradually to within normal limits

These observations on urobilinogen excretion in cases of Addison's "pernicious" anemia go to show (1) that in the state of relapse there is a markedly increased blood destruction, (2) that adequate liver treatment controls this destruction, (3) that the rapid fall in urobilinogen excretion, beginning during the reticulocyte response, suggests that the abnormal red blood corpuscles present in a relapse are the ones chiefly affected in the stage of increased blood destruction

Some Effects of Adrenalin on the Heart with Especial Reference to Its Use As a Test for Angina Pectoris By S A LEVINE, and (by invitation) A C ERNSTENE and B M JACOBSON Boston, Mass

Observations by one of us (S A. L) on a patient with bronchial asthma and angina pectoris in whom an attack of angina was precipitated by a therapeutic dose of epinephrine gave rise to the idea that possibly epinephrine could be employed as a diagnostic measure in patients in whom the diagnosis of angina pectoris was not certain. With this in mind, one cubic centimeter doses of a 1 1000 solution of epinephrine chloride have been given subcutaneously to eleven patients with angina pectoris and in all but one a typical anginal attack has been produced The single exception cannot, for various reasons, be accepted as a complete failure In some instances the pain was allowed to subside spontaneously, while in others it was brought to an end by nitroglycerine or amyl nitrite Ten patients of the same age group as those with angina pectoris but without evidence of cardiac disease and ten normal young adults have been tested similarly, and in no instance has anginal pain been produced

Frequent blood pressure determinations and electrocardiograms were taken in each experiment. It was originally hoped that distinctive changes might be found in the electrocardiograms of anginal patients as a result of the epinephrine, which might further serve as a diagnostic aid In only one case, however, did any important changes develop although extrasystoles were frequently noted There was nevertheless a striking difference in the response of the T wave in the three

groups   In the patients with angina pectoris, the T-wave invariably increased in amplitude, while in the group of older controls there was, as a rule, very little change in amplitude, and in the young controls there was usually a decided diminution in height of the T-wave   This work indicates that adrenalin may serve as a diagnostic test for angina pectoris   Its use, however, would need to be applied with caution and discretion

Emphysema Simulating Cardiac Decompensation   By W B KOUNTZ (by invitation) and H L ALEXANDER, St Louis, Mo

Sixty-three cases of advanced emphysema were studied   In all, there were dyspnea on exertion, and some cyanosis, and in forty, dependent edema   Direct examination of the cardiovascular system showed no abnormality excepting consistently increased venous pressure

Autopsies were performed in nine cases   Eight of these had had dependent edema   No cardiac lesions appeared   The right ventricular wall was not increased either in thickness or in weight

Dyspnea in these cases is attributed to the consistently lowered vital capacity, cyanosis, to deficient pulmonary ventilation   The dependent edema is presumed to be caused mainly by increased venous pressure

The rise in venous pressure in these cases was studied experimentally, and found to be due to two factors, increased intrathoracic pressure and anoxemia

An extensive emphysema in dogs was produced by inserting an adjustable ball-valve in the trachea   During the development of the emphysema, periodic intrathoracic and peripheral venous pressures were taken simultaneously   It was found that the venous pressure rises proportionately to the increase in intrathoracic pressure   Dogs were rendered partially anoxemic and a rise in venous pressure in proportion to the degree of the oxygen unsaturation of the arterial blood was observed

These observations tend to refute persistent statements that emphysema leads to right heart failure

Chronic Infectious Non-Tuberculous Pulmonary Lesions of Childhood and Their Relation to Bronchiectasis   By F MAURICE MCPHEDRAN, Philadelphia, Pa

A number of these lesions have been observed in children during the past six years   Some were seen shortly after an illness severe enough to initiate a lesion, many during recurring attacks of pneumonia from which a persisting infiltration results, others, without symptoms, were discovered by routine x-ray examination   The last usually sooner or later suffer characteristic bronchopneumonic exacerbations, which are roentgenographically recordable, sometimes producing severe illness, sometimes accompanying apparently trivial coryza

The lesions predominate in the left lower lobe   When early, whether right or left, they are densest close to the heart   Synchronized diastolic roentgenograms accurately record slight lesions and eliminate misleading blurring due to systole

Early diagnosis and care is important to prevent advanced scarring and bron chiectasis

Among 106 colored children occurred five cases, among 325 Italians 19, among 341 other white children only 4 Five cases occurred in one family, three in each of two others In two additional families each presenting two cases the fathers had suffered from clinical sinusitis for many years

These lesions are commonly diagnosed tuberculosis Differentiation presents few difficulties

No characteristic or predominating organism has been found Paranasal sinusitis is common

Clinical and Experimental Studies of the Effect of Ergotamine on Metabolism and the Circulation By JOHN B YOUMANS, M D , and (by invitation) W H TRIMBLE

Ergotamine tartrate in doses of 0 5 mgm. subcutaneously caused a small drop in pulse rate and a small rise in diastolic pressure in normal human subjects under basal conditions No significant effect on metabolic rate or blood sugar was no ticed over periods up to three hours Severe muscle pains and soreness, nausea, headache and lassitude were common Atropine in doses of 1.2 mgm subcutane ously prevented or abolished the drop in pulse rate Continued administration of ergotamine in doses of 1 mgm by mouth, three times daily for a week had no significant effect on metabolic rate Patients with thyrotoxicosis showed in general similar results but the effect on pulse rate and blood pressure was much more marked

Doses somewhat larger (0 105 to 0.5 mgm per animal), but smaller than those usually reported, were employed intravenously in trained unnarcotized dogs In these animals under basal conditions ergotamine caused an immediate and marked fall in heart rate which was prevented or abolished by atropine No constant changes in oxygen consumption or blood sugar levels were observed over periods up to three hours In the doses used ergotamine failed to prevent or diminish epinephrine hyperglycemia in these animals

Observations on Changes in Respiration and Circulation Occurring Coincidentally with Sensations of Faintness and Impending Syncope in Normal Individuals By ISAAC STARR, JR and (by invitation) LEON H COLLINS, JR., Philadel phia Pa

While there has been much speculation concerning the physiology of syncope (Gowers, Hering, etc.), observations upon this condition have been limited to changes in pulse rate and blood pressure, chiefly after collapse (Cotton and Lewis) The opportunity to extend these observations came during experiments designed to determine the applicability of the ethyl iodide cardiac output method (Starr and Gamble) to the problem of the effect of posture on blood flow The subjects, standing, inhaled ethyl iodide, blood being obtained from a vein on the back of

one hand immersed in water at 45°C   A graphic record of respiration was taken from the spirometer, blood pressure and pulse rates were observed at frequent intervals

Four subjects experienced faintness and two collapsed   The records show that the sensation of faintness was always accompanied by hyperventilation but was usually without significant change of pulse rate, blood pressure or cardiac output Symptoms of collapse were preceded by marked hyperventilation and accompanied by marked fall in pulse rate and blood pressure and apparent diminution of ventilation   Alveolar CO_2 was very low in two such cases, in one of which improvement followed rebreathing   The cardiac output could not be determined at the time of collapse, it remained unchanged or fell but little during the period preceding

Certain features of the physiological picture described   low blood pressure without diminution of cardiac output have been produced in anesthetized cats (Dale and Evans) by hyperventilation by blowing off of CO_2, but no pronounced fall of heart rate occurred in these experiments   Other features, hyperventilation and slow pulse regularly occur directly after the production of cerebral anemia in animals (L Hill, Pike, Schmidt, etc), but a high rise of blood pressure usually occurred at this time   In an occasional experiment in this laboratory, occlusion of the cerebral arteries of an anesthetized cat caused prolonged hyperventilation and an elevation of blood pressure lasting only a few seconds followed by marked slowing of the pulse rate with fall of blood pressure below the initial level   These results seem essentially similar to the physiological picture we observed in impending syncope in normal individuals

The fact that the hyperventilation precedes the fall of blood pressure and pulse rate in our subjects is evidence against the conception (Lewis) that the primary cause of syncope is reflex vagus stimulation   Our observations are consistent with the conception that syncope is caused by cerebral anemia secondary to failure of the peripheral circulation to adjust itself to the upright position and so maintain the blood supply to the brain

Skin Reactions of Patients and Normal Individuals to Protein Extracts of Streptococci

By CLIFFORD L DERICK and (by invitation) MARSHALL N FULTON, Boston, Mass

Skin tests were carried out on 366 general hospital patients, 67 infants and children in a general medical ward and 51 patients with rheumatic fever or chorea in whom the disease was either active or early in the convalescent stage   A group of apparently normal adults were similarly tested for comparison

The purpose was to determine what proportion of these individuals would show skin sensitiveness of the tuberculin type to protein extracts of different varieties of streptococci   Test materials used were pure nitrogen-constant extracts from each of three types of streptococci—hemolytic, green-producing, and indifferent A similarly prepared extract from Baker's yeast was used for control purposes Varying amounts in normal saline solution were injected into the skin of the forearm   All readings were made at 24-hour intervals for 2 or more days

The analyzed results reveal that patients with certain diseases show a high incidence of positive reactions, that there seems to be a relationship between previous infections and skin sensitiveness, and that age, except in the very young, plays a minor if any rôle

The presence of reaction is looked upon as indicating present or previous infection with streptococci and is not characteristic of any one disease

Rate of Removal of Bacteria from the Blood in Human Disease   By REUBEN OTTENBERG, New York, N Y

A technic has been developed by which simultaneous blood cultures are made from the two internal jugular veins   In sinus thrombosis there is usually a much larger number of colonies in one vein than in the other   In 20 of the more recent cases a simultaneous blood culture was also made from an arm vein   I wish at present to point out the very small number of colonies usually found in the arm vein as compared with that jugular vein which is draining the lesion most directly

Among 17 cases of otitic infections suspected to be sinus thrombosis there were 7 in which the colonies could be counted   In every one of these the number of colonies in the arm blood was much smaller than in one or both jugulars   Thus

Colonies per cubic centimeter of blood

	Right jugular	Left jugular	Arm vein	
Case 4	122	7	6	Left sinus thrombosis
Case 25	600	11	1	Right sinus thrombosis
Case 32	250	240	1/3	Right sinus thrombosis

In two cases of ulcerative endocarditis the number of colonies found in the jugular veins and arm vein were approximately equal.

It is evident then that during circulation of blood through the lungs an enormous number of bacteria are removed   The number in the periphery represents only a small fraction of those which are constantly being fed into the blood stream

White Blood Cells in Medical Conditions   By PAUL REZNIKOFF, New York, N Y

The object of this study was to determine variations of the white blood cells and the intensity and course of "the shift to the left" (the response of young polymorphonuclear cells) during the course of illness   Total white cell and Schilling counts were performed on 177 patients on the medical wards.

These included 78 patients suffering from pneumonia and 23 having some form of upper respiratory infection   Four tabulations were made (a) total white cell counts, (b) percentage of polymorphonuclears (c) percentage of all cells which are young polymorphonuclears, (d) percentage of all polymorphonuclears which are young

It was found that all patients suffering from acute infections have a shift to the

left regardless of their total white blood, total polymorphonuclear count or temperature   The increase in the relative and absolute number of young polymorphonuclears varies roughly with the intensity of the infection   The return of the shift to the right or failure to do so is the most accurate gauge of prognosis that can be obtained from blood studies

Arthritic Pain in Relation to the Weather   By L G ROWNTREE and (by invitation)
     E B RENTSCHLER and F R VAN ZANT, Rochester, Minn

For a period of a year we have studied the pain in a series of arthritic patients, 377 in all   We have found a striking relationship between the pain and the presence of storms about 90 per cent of the time   Between barometric pressure and pain we have found a relationship 93 per cent of the time   In 72 per cent there has been a direct relationship, and in 21 per cent a negative, or inverse relationship   Sunshine seems to be another factor of considerable interest   We have also studied a group of arthritic "weather prophets" and found that many of them can predict weather changes 12 to 24 hours in advance   The literature has been covered in connection with this subject, but little of an exact nature has been written to date   The possible relationship of the sympathetic nervous system to this phenomenon has been introduced through the discussion of a patient in whom removal of the second, third and fourth lumbar sympathetics was done   This patient was suffering from arthritis and had always noticed a relationship between storms and increase of her pain   After the operation there was no such relationship in the lower extremities, while the upper extremities, which were also afflicted with arthritis, continued to be affected by storms

The Respiratory Quotient of the Obese During Reduction   By H B McCLUGGAGE,
     and J M STRANG, (by invitation) and FRANK A EVANS, Pittsburgh, Pa

Observations made on ten obese patients undergoing reduction by dieting during an average period of fourteen weeks showed a basal R Q of less than 0 707 in 35 per cent of 112 determinations   The average R Q before dieting was 0 776 for 8 subjects, the average R Q during the dieting period was 0 728   Very low respiratory quotients appeared usually after two weeks of dieting   Only one patient failed to go below 0 707 at some time   *The average respiratory quotient reaches its lowest level during the third month when 19 determinations on 7 patients averaged 0 701*

The lowest observed R Q 0 632, was obtained at the eighth test on a patient on whom 6 of 13 tests ran low   Another patient, on whom 13 of 26 determinations were low, had values of 0 653, 0 650 and 0 657 on three successive weeks and a minimum of 0 640   The average of the lowest respiratory quotients observed on 9 patients was 0 661

These very low respiratory quotients do not correspond to (1) the periods of most rapid weight loss, or (2) the presence or absence of acetone bodies in the urine   A large number of blood CO_2 combining power determinations have fallen within normal limits

Observations Concerning Intracranial Circulation in the Human Subject
WILLIAM G LENNOX, Boston, Mass

The oxygen and carbon dioxide content of arterial blood and of venous l
from the internal and external jugular and the basilic veins was measured
number of patients   In these subjects, the gaseous content of blood from
external jugular vein approached that of arterial blood   In many of the ind
uals there was a wide discrepancy between the gaseous content of blood from
internal jugular and the basilic veins   The average measurements for the v
group, however were about the same

In experiments conducted with Dr Soma Weiss, it was observed that th
travenous injection of histamine caused the venous blood from each of these
veins to become more arterial like   Apparently cerebral vessels respond to
stimulation in much the same way as vessels of the skin

The Cerebral Circulation   \ The Action of Histamine   By H S FORBES
H G WOLFF (by invitation) and STANLEY COBB, Boston, Mass.

By the method developed by one of us for observing the pial vessels throu
window in the skull, studies have been made of the effect of histamine on
cerebral circulation.   It was observed that the vessels of the brain react to i
venous injection of histamine in a very different manner, depending on wh
the animal is under ether or amytal anesthesia.   Under ether the vessels o
brain are already dilated and so do not dilate to any extent, if at all follc
intravenous injection of histamine   On the contrary they often become narr
and the cerebrospinal fluid pressure falls.

Local application of histamine to the surface of the brain always resul
dilatation of the pial vessels, without noticeably affecting intracranial or syst
vascular pressures   Intravenous injection of histamine (in animal s under :
tal) causes great pial artery dilatation in spite of a coincident fall in syst
arterial pressure

Studies in the Etiology and Iodine Reactions of Simple Goiter in Rabbits
BRUCE WEBSTER (by invitation) and ALAN M CHESNEY, Baltimore M

Further studies have been carried out to determine the etiology of an epid
of simple goiter in rabbits   These suggest that the basic factor in this insta
a dietary one   The addition of 7 5 mgm. of iodine per week to the goiter pro
ing diet has been found sufficient to prevent the development of goiter   Imp
ing the sanitary conditions under which the animals were kept, by altering
manner of caging so as to reduce the fecal contamination of the food, was f
to exert only slight protective action against the goiter producing influence

Previous studies have shown that the administration of iodine to goit
rabbits is followed by a marked increase in heat production   Studies have
made in an effort to quantitate the production of thyroid hormone against a
able iodine   Rises in heat production were studied after the administration

left regardless of their total white blood, total polymorphonuclear count or temperature   The increase in the relative and absolute number of young polymorphonuclears varies roughly with the intensity of the infection ' The return of the shift to the right or failure to do so is the most accurate gauge of prognosis that can be obtained from blood studies

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or paroxysmal than that due to other causes   One fourth of the cases in this series were of this type

The cardiac manifestations of Graves' disease may dominate the picture and obscure the underlying thyroid disorder, they may even antedate all other recognizable signs of the Graves' disease causing them

Digitalis is less effective than in fibrillation not due to Graves' disease   The amounts tolerated are larger, and the beneficial effects are less striking

Following successful treatment of the underlying Graves' disease, recovery from fibrillation is usually prompt and striking   Restitution of the heart to normal is often complete   Quinidine is often very useful in restoring permanent normal rhythm

Cardiac hypertrophy is seldom pronounced   Enlargement, when present, is due chiefly to dilatation

In some respects the cardiac disturbances of Graves' disease resemble those of toxemia of acute infection

Although it is difficult to estimate the relative importance of mechanical and toxic factors, the infrequency of hypertrophy and the character of the disturbances suggest that the latter is of major importance

A Comparison of the Coronary and Digitalis T Waves   An Electrocardiographic Study   By WALTER W HAMBURGER, Chicago, Ill

Inversion of the T wave of the human electrocardiogram, is one of the most frequent findings in clinical electrocardiography   The two most frequent causes of T wave inversion are coronary disease and digitalization.   The character of inversion is different in these two conditions   coronary inversion shows the typical cove shape (Pardee)   digitalis inversion appears as an immediate straight line depression of the R T interval   A coronary-digitalis wave appears as a merging of the two   Examples of these three types of inversion are presented   The clinical importance of recognition of these various types is emphasized

Skin Tests with Pneumococcus and B influenzae Antigens in Influenza and Pneumonia   By ALLAN K POOLE, and JOHN H BUMSTEAD, (by invitation) and FRANCIS G BLAKE, New Haven, Conn

The study was undertaken with the purpose of determining the skin reactivity or allergy of patients with uncomplicated influenza and with pneumonia to antigens prepared from Pneumococcus Type I, Pneumococcus Type II and *B influenzae*   Patients with influenza (23 cases) consistently showed marked reactivity to all three antigens in the acute febrile stage   lessened reactivity during the first week of convalescence and in the majority no reactivity after the 20th day   Patients with pneumonia (32 cases) showed a striking contrast.   With one exception, a case of streptococcus pneumonia, no case reacted to the pneumococcus antigens during the acute stage, but those retested became reactive during convalescence   Forty per cent reacted to *B influenzae* antigen during the acute

single small doses of potassium iodide to animals in which the degree of hyperplasia of the thyroid gland had been previously determined by biopsy   The increase in heat production appeared to vary directly with the amount of iodine exhibited, as did also the extent of involution of the gland   This relationship apparently held true until involution was complete

The Effect of Liver and of Liver Extract upon Appetite   By A C CURTIS (by invitation) and L H NEWBURGH, Ann Arbor, Mich

The addition of 2 per cent protein as dried ether extracted liver to a basic rat diet containing 2 per cent vitamine B powder (Harris) causes greater energy ingestion and better growth than does the addition to this diet of 2 per cent protein in the form of casein

If vitamine B powder is omitted from both diets, greater loss of appetite and weight occurs in the group of rats eating the basic diet containing 2 per cent protein in the form of casein   All animals of both groups develop polyneuritis

The addition of 2 per cent liver extract (Lilly) to the basic rat diet plus one yeast vitamine powder tablet daily (Harris) causes much greater ingestion of energy and much better growth than was observed when 2 per cent dried ether extracted liver protein or casein protein was added to the basic rat diet

When the basic diet, containing added liver extract (Lilly), ether extracted liver protein, or casein protein, is supplemented by sufficient yeast vitamine powder (Harris), energy ingestion and growth are equal

If basic rat diets, containing autoclaved ether extracted liver and liver extract, are fed without yeast vitamine powder, polyneuritis develops in all experimental animals

When 2 per cent liver extract (Lilly) is added to the basic diet, which contains no other source of vitamine B, growth and energy ingestion are approximately normal   The replacement of liver extract by equal amounts of yeast vitamine powder (Harris) causes less growth and less energy ingestion

A patient with pernicious anemia was fed 24 grams of autoclaved liver extract (Lilly) daily for 6 days and did not experience a rise in reticulated cells or red cells   At the end of this period 24 grams of unaltered liver extract (Lilly) was fed daily with a reticulated cell rise and red cell rise occurring in the usual time

1   The factor in liver that effects appetite is, seemingly, that fraction of vitamine B

2   Liver extract (Lilly) appears to contain the appetite factor of vitamine B in concentrated form

Auricular Fibrillation in Graves' Disease   By PAUL S BARKER, and ANN BOHN-ING (by invitation) and FRANK N WILSON, Ann Arbor, Mich

A study of 107 unselected cases of auricular fibrillation due to Graves' disease, and observed from 1923 to 1927, leads to the following conclusions

The auricular fibrillation due to Graves' disease is more likely to be transient

higher percentage than normal of abnormal sized red blood cells in the peripheral blood of patients having carcinoma of the breast or prostate, and with a normal blood count suggests the possibility of bone marrow metastasis   Anemia from other causes must be eliminated when the red blood cell count is lower than normal before the abnormal sized cells can be considered as suggestive of bone metastasis, although cells larger than normal are of greater significance

A Comparison of Volume Index and Diameter of the Red Cells in Different Types of Anemia   By RUSSELL L HADEN, Kansas City, Kans

Accurate knowledge of the size of the red cells is of fundamental importance in the study of the anemias   The size of the average cell may be determined from the volume index which gives the volume of the average cell   The size may also be determined by measuring the diameter of a representative group of cells   Fifty cases each of pernicious anemia and of secondary anemia have been studied by these two methods   The data obtained and the advantages and disadvantages of the two methods are discussed

The Measurement of Temperatures in the Bladder, Cervix and Rectum of Patients Receiving Diathermy Treatment of the Pelvis   By F W BISHOP and E K RITCHIE, (by invitation) and S L WARREN, Rochester, N Y

The pelvis was treated by passing a high frequency or diathermy current between a speculum in the vagina and a sacral plate.

Temperatures were measured by thermocouples placed in the cervix, bladder, rectum and elsewhere

When the high frequency is turned on, all of the regions measured showed a simultaneous rise in temperature to a plateau which was maintained while the current was applied   On turning off the current there was a similar drop in the temperatures to the original value   Each state of equilibrium was reached in about five minutes

With currents of from 1800 to 2400 milliamperes in average patients, local temperatures as high as 44°C were measured and maintained at this level during treatment   The temperatures in the rectum cervix and bladder were very nearly the same   Individual patients varied considerably in the amount of increase in temperature found the average attained being around 42°C   Body temperature (mouth) was elevated only 0.5° to 1 0°C   Blood pressure was unaffected

The Question of Arsphenamine Resistant Syphilis   By JOSEPH EARLE MOORE, and (by invitation) HARRY M ROBINSON, Baltimore, Md.

A survey of the literature indicates that in Germany and perhaps elsewhere in Europe, early syphilis reacts to arsphenamine treatment less favorably now than in the years before the War in the sense that secondary relapses are more frequent, the blood Wassermann is not so rapidly reduced to negative, and an increasing number of patients whose lesions fail to heal under arsphenamine treatment are

stage   Those retested in convalescence retained or developed reactivity   In brief, patients with influenza appear to show heightened reactivity to bacterial antigens during the acute stage and tend to lose this after recovery, patients with pneumonia show little reactivity during the acute stage and tend to become more reactive after recovery   The interpretation awaits further studies now being carried on

Venous Pressure in General Anaesthesia   By O O MEYER (by invitation) and
    WM S MIDDLETON, Madison, Wis
    Forty-two individuals under general anaesthesia have been studied with a view to determining the influence of such agents   The results may be summarized as follows
    1   Coincident with the increased muscular effort and altered respiratory function of the induction period, pronounced increases in venous pressure are noted
    2   Thereafter a subsidence occurs to a plateau of venous pressure somewhat elevated above the normal, which is maintained throughout the maintenance of anaesthesia
    3   Alterations in this plateau apparently depend upon reflex stimuli, usually in a period of lightened anaesthesia
    4   Elevations in venous pressure occur on release from anaesthesia dependent upon such acts as retching, vomiting, etc
    5   Carbon dioxide inhalations operate apparently independently to increase venous pressure
    These results are not interpreted as bearing a causal relation to the circulatory accidents of general anaesthesia and surgical operation   Indeed, the demands of ordinary life with its occasional calls for sharp elevation in venous pressure, as in the act of defecation, may lead to much greater strain on the right heart than has been noted in these venous pressure determinations under properly administered general anaesthetics

*Red Blood Cell Measurements in the Diagnosis of Metastatic Cancer in the Bone
    Marrow*   By RAPHAEL ISAACS and (by invitation) MADELAINE R BROWN,
    Ann Arbor, Mich
    In patients with carcinoma with metastasis to the bone marrow, red blood cells smaller or larger than normal appear in greater frequency in the blood stream than in normal persons   Seven out of thirteen patients showed a disproportionate percentage of cells larger than normal and all of them showed a relative increase in the percentage of cells smaller than normal at some stage in their disease   In no case did the degree of microcytosis approach that of the blood after hemorrhage or in secondary anemia from other cause   With the present data no evident correlation between the degree of variation in the size of the cells and the extent of the metastasis, can be made, due, probably, to the difficulty in judging the true degree of the marrow involvement   The presence of a relatively

higher percentage than normal of abnormal sized red blood cells in the peripheral blood of patients having carcinoma of the breast or prostate, and with a normal blood count suggests the possibility of bone marrow metastasis. Anemia from other causes must be eliminated when the red blood cell count is lower than normal before the abnormal sized cells can be considered as suggestive of bone metastasis, although cells larger than normal are of greater significance.

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appearing   It seems probable that this situation is due less to any decrease in the therapeutic activity of arsphenamine, or to the development of so-called arsphenamine-fast strains of spirochetes, than to the reaction of the individual patient to treatment   Composite Wassermann curves of 1116 patients with early syphilis studied from 1914 to 1927 in the Syphilis Clinic of the Johns Hopkins Hospital show that there is no significant difference in serologic results in recent as compared with earlier years   Relapses of a secondary type are, in our material, no more frequent in 1925–1927 than in 1914–1923   True arsphenamine-resistant syphilis is not increasing in this locality   Patients with lessened resistance to syphilitic infection as measured by the standards of "seronegative early secondary syphilis" or "premature Wassermann negativity" are no more frequent in our material now than in earlier years   There is no evidence to indicate that, in this part of the United States, arsphenamine is less efficacious than formerly in the treatment of syphilis, or that arsphenamine resistant syphilis is increasing   Certain general aspects of the phenomenon of arsphenamine-resistance are discussed

The Relation of Recovery and Development of Humoral Immunity in Pneumococcus Pneumonia in Children   By C O'DONOVAN, JR , (by invitation) and JAMES D TRASK, New Haven, Conn

In fixed type pneumococcus pneumonia in adults recovery has been shown to be associated with the development of type specific humoral antibodies

Children are supposed to recover from this disease more regularly than adults Accordingly a study was made of the development of type specific agglutinins in the blood serum of 19 children with the disease   There were 12 Type I, 2 Type II and 5 Type III cases, all recovered   However the development of specific antibodies was found to be significantly less marked than has been reported for adults

The Tissue Transfer Method of Determining Cure of Syphilis in Man   By HUGH J MORGAN and (by invitation) JAMES L ALLOWAY, Nashville, Tenn

Brown and Pearce have demonstrated the frequency with which treponemas localize in the lymph nodes of infected rabbits and remain there long after the acute stage of the infection has passed   This lymph node reservoir for treponemas is used by workers in experimental syphilis as a means for recovering organisms from animals without active lesions   This may be readily done by emulsifying a lymph node and injecting it into the testicle of a normal rabbit   An acute orchitis develops in the inoculated animal or in subsequent transfers   After treatment of experimental syphilis in the rabbit, failure to recover organisms by this method has been interpreted as an indication of cure

In 1927 Chesney and Kemp stimulated interest in the possibility that the tissue transfer method of determining cure of experimental syphilitic infection might be applicable to syphilis in man   They applied the method to three patients and

were unable to recover treponemas from lymph nodes after suitable treatment. This work raised the very important question as to whether or not these negative results indicate biological cure

In order to throw some light on this subject we have undertaken a study of the recoverability of treponemas from lymph nodes of untreated patients in different stages of the disease   It was felt that if, in untreated cases of syphilis, as in the experimental disease, treponemas could always be recovered from lymph nodes, then the positive or negative findings after treatment would be of great significance and might be used as criteria of cure

In all instances enlarged lymph nodes (inguinal, epitrochlear or cervical) were removed under novocaine anesthesia, immediately cut into small bits and ground in a mortar with a small amount of warm saline solution   The resulting emulsion was injected in equal amounts into the right testicles of two normal rabbits   The animals were kept under constant observation.   Dark field examinations were made with specimens from all visible or palpable scrotal or testicular lesions and with all subsequent testicular emulsions used for transfers   Animals which developed no lesions at the end of ninety days were sacrificed and the right testicle of each was removed, emulsified, examined by dark field and injected intratesticularly into two normal rabbits   No tissue transfer experiment was declared negative until transfers had thus been made through two sets of rabbits.

Our findings are summarized in the following table

TABLE 1

The recovery of treponemas from lymph nodes in untreated syphilis (tissue transfer method)

History number	Clinical diagnosis	Tissue transfer	
11238	Primary syphilis	Positive	
11806	Primary syphilis	Positive	
11405	Secondary syphilis (late)		Negative
9655	Latent syphilis		Negative
11569	Latent syphilis (congenital?)		Negative
11890	Tertiary syphilis (leg ulcers)		Negative
12227	Tertiary syphilis (skin recurrence)	Positive	
10987	Tertiary syphilis (gumma of knee).		Negative
10829	Neurosyphilis (tabes dorsalis)		Negative

We conclude from this study that the tissue (lymph node) transfer method of determining the presence or absence of syphilitic virus in experimental infection does not invariably give positive results when applied to patients with proven untreated syphilis   Therefore, in human syphilis the method is unreliable   In a given case negative results before any treatment is instituted is of no diagnostic significance.   After treatment is instituted negative results cannot be interpreted as indicating biologic cure   It is suggested that this discrepancy in results with the tissue transfer method in human and experimental syphilis is probably due to

the fact that in the latter the virus is usually one thoroughly adapted to the rabbit by previous animal passage   Dosage also is probably important and this factor is now being studied by us

Observations on the Apparent, Adaptability of the Body to Infection, Unusual Hardships, Changing Environment and Prolonged Strenuous Exertion   By BURGESS GORDON and (by invitation) JOHN C BAKER, Philadelphia, Pa

Competitors in a transcontinental foot race (March, April and May, 1928) who walked or ran 3482 miles in 84 consecutive days were studied before, during and after the contest   In histories obtained from 174 contestants a wide variation in age, physical condition, preliminary training and diet was noted   Infections, injuries and experiences common in frontier life of 75 years ago occurred during the race   The chief causes for withdrawal from the contest were so-called "shin splints," resulting from myositis and periostitis   Various fads and fancies in diet were advocated by the runners at the beginning of the race   As the contest progressed, however, meat and certain vegetables could not be supplied as desired   Carbohydrate became the predominant food   Several competitors consumed no meat during the entire race and others none after the first 500 miles   It was noted that carbohydrate in the form of oranges, dates, figs, honey, dextrose and cake between meals was taken by most runners after the first few hundred miles, apparently in order to relieve hunger and fatigue

Observations made after the race, which included microscopic and chemical studies of the blood and urine, roentgenographic, electrocardiographic, ophthalmoscopic, orthopedic and laryngeal examinations were essentially negative as would be expected from a group of apparently normal individuals who had followed less strenuous forms of exercise   Correspondence during the past eight months shows that 84 contestants have suffered no apparent untoward effects

In considering the condition of the runners at different periods during the race it would appear that it is extremely difficult to estimate the potential endurance of an individual or to predict the degree of resistance to infection

A Clinical Metabolic Study of "Obstinate" Cases of Obesity   By SOLOMON STROUSE and (by invitation) C C WANG, Chicago, Ill

This work was undertaken on patients who had resisted supposed strict dietary procedures at home   They were then established in the metabolic clinic at the hospital and placed under complete control   A noticeable contrast was shown and the patients could be made to lose weight mathematically according to schedule

The Clinical Interpretation of Diabetes   By JOHN R WILLIAMS, Rochester, N Y

The following is an abstract of a study of upwards of five hundred diabetics with reference to a better means of control and interpretation   It is commonly believed that insulin causes the burning or utilization of 1 5 to 2 grams of glucose

per unit   For the past six years the writer has coöperated with the Eli Lilly Company in the clinical testing of insulin   In this work one patient, whose metabolism is fairly constant, and who is highly trained has been used as a test case   The results obtained have been carefully checked and have invariably corresponded closely with the assays of the Lilly Company   The work which will be reported in a later communication indicates that one unit of insulin causes the utilization of approximately 4 grams of glucose rather than 2 grams as is commonly assumed

The diet of normal individuals contains from 300 to 800 grams of glucose forming food   One unit of insulin will burn approximately 4 grams of glucose   The normal pancreas makes from 75 to 200 or more units of insulin daily according to the food intake of the individual   When the pancreas fails to make sufficient insulin to care for the glucose content of the diet diabetes results   That portion of the glucose which is not utilized and which exceeds the storage capacity of the body will be eliminated as urine sugar   The difference between the amount of glucose ingested and that excreted in the urine is the glucose utilization of the patient   The glucose utilization factor in a well treated case of diabetes is fairly constant and may be increased by the administration of insulin   In either a normal individual or a diabetic the number of grams of glucose utilized, divided by four, will give the number of units of insulin made by the individual   This figure hereafter designated as the insulin coefficient is a fairly constant factor in the diabetic.   Example—The diet contains 100 grams of glucose, 20 grams are eliminated in the urine, therefore 80 grams are utilized   The pancreas makes 20 units of insulin

To determine the coefficient of a patient taking insulin, the number of units administered must be subtracted from the total number of units required for the utilization of the food   Thus in the above case if the patient were receiving 8 units daily his coefficient would be 20 minus 8 or 12   Written IC 12   The insulin coefficient is a fairly constant factor and changes slowly with improvement or decline   It is not materially affected by occasional violations in diet even in the presence of large amounts of urine sugar and high blood sugar levels, nor is it influenced by insulin administration

A severe diabetic is one whose insulin coefficient ranges below 20, moderately severe cases range from 20 to 35 mild cases from 35 to 75   A diabetic may have a minus coefficient   Example The diet contains 100 grams of glucose, the urine 20 grams of sugar 30 units of insulin are being administered daily   Insulin coefficient is −10   There are several explanations for this minus coefficient   (a) The insulin may be administered in too large a dose, in excess of the body requirements at a given time and is eliminated in the urine, without serving any useful purpose, or (b) an infection or some other unsuspected disease may be present which destroys insulin, or (c) there may be an error in insulin administration or (d) in diet   The insulin coefficient is a more valuable guide to the progress of an infection in diabetes than is the temperature curve or the leucocyte count   It has

great prognostic value   It affords a most reliable check on the integrity of the patient who violates his diet or insulin dosage   It is by far the best method of measuring the progress of a case and the efficiency of the treatment   A patient who can raise his coefficient from 5 to 10 units in a year is making satisfactory progress

In making tests to determine the insulin coefficient, the patient should be thoroughly instructed as to its significance and the necessity for exact cooperation The diet should be constant for at least three days if possible   The urine should be saved and measured with care   Urine sugars should be accurately determined A slowly rising midmorning blood sugar indicates that the patient is gradually exceeding his capacity to utilize glucose   The insulin coefficient is a far better method of differentiating spurious diabetes and renal diabetes from true diabetes than is the so-called glucose tolerance test   A glucose tolerance test is about as useful and reliable a method of determining the efficiency of the pancreas as is the "hundred yard dash in eleven seconds" a measure of the physical ability of an individual to walk five miles at a leisurely gait

At the present time there is no clinical method in use which enables clinicians in one community to compare the efficiency of their methods with the clinicians of another community   Likewise there is no clinical method in use which enables physicians to accurately contrast or evaluate different therapeutic measures The insulin coefficient method meets these requirements

Paroxysmal Bundle Branch Block   By BENJAMIN M BAKER (by invitation) and
    EDWARD P CARTER, Baltimore, Md
An example of paroxysmal bundle branch block will be presented.   The patient, when first seen, showed paroxysmal auricular fibrillation and paroxysmal bundle branch block   It was noted that during the periods of delay in intraventricular conduction the cardiac rate was above 85 to the minute   When the rate fell below this figure normal sequential rhythm with normal intraventricular conduction occurred

Under rest and the administration of digitalis normal sequential rhythm with normal intraventricular conduction was established which constantly altered to bundle branch block when the cardiac rate was appropriately increased by various measures, thus indicating that, at least, one control of the spread of the excitation wave was the auricular rate   Delay in intraventricular conduction occurred both with and without fibrillation of the auricles

Several galvanometric records will be shown portraying in a single lead transition from normal sequential rhythm with normal intraventricular conduction to bundle branch block

Studies are now in progress to determine the part played by anoxemia in intraventricular conduction

THE INFLUENCE OF THE SYMPATHETIC NERVOUS SYSTEM ON THE CAPILLARIES DURING PASSIVE CONGESTION

By J HAMILTON CRAWFORD

(From the Departments of Physiology and Medicine, Long Island College Hospital)

With the discovery of vasomotor nerves by Claude Bernard the importance of the autonomic nervous system in circulatory regulation came to be recognized   The action of the sympathetic nerves on the arteries and arterioles was definitely established but it has taken many years for any action by them on the capillaries to become accepted Indeed the capillaries were considered merely passive endothelial tubes whose variation depended on changes which took place in the other parts of the vascular system   In 1860 Beale (1) described nerves accompanying the capillaries and Glaser (2) considered these to be sympathetic nerves   The connection of these nerves to the vessel wall, however, still remains uncertain   Among the early papers Steinach and Kahn (3) presented the most positive evidence that nerve stimulation could influence the capillaries independent of its action on other vessels   They showed that stimulation of the third, fourth and fifth thoracic nerve roots at their exit from the cord produced emptying of the capillaries in the nictating membrane of the frog when the circulation was at a standstill   Krogh, Harrop and Rehberg (4) confirmed this by stimulating the lower ganglia (8–10) of the sympathetic in the frog while studying the vessels in the web under the microscope   They found that the arterioles contracted first and a few seconds later the capillaries contracted independently   The first evidence of capillary contraction following nerve stimulation in mammals was presented by Hooker (5), who stimulated the cervical sympathetic in the cat while he watched the capillaries in the depilated ear under the microscope   He observed a contraction of the capillaries independent of that of the arterioles The most conclusive evidence of the independent contractility of the

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capillaries following nerve stimulation is that brought forward recently by Harris and Marvin (6)   They studied the effect of stimulation of the cervical sympathetic on the vessels in the ear of the albino rabbit   They were able to confirm Hooker's observations but they also showed that stimulation of the sympathetic caused emptying of the capillaries when the circulation was brought to a standstill by obstructing the arterial supply   Under the conditions of their experiment the effect of contraction of the arterioles or venules on the size of the capillaries would obviously have been to distend them, and not to cause them to disappear   As the result of the investigations described there can be little doubt that the sympathetic nervous system can cause contraction of the capillaries independent of its action on the other parts of the vascular system

The evidence for a supply of vasodilator nerves to the capillaries is much less certain   Doi (7), Krogh, Harrop and Rehberg (4) and Langley (8) (9) have studied this problem and believe that such exist   They all used frogs, except Langley who used the cat's paw, and found that peripheral stimulation of posterior root ganglia or sensory nerves produced vasodilation   They endeavored to rule out the action on the arterioles by various means, Doi by dilating the arterioles with acetylcholine and Langley by occluding the circulation before applying the stimulus   Lewis and Marvin (10) repeated Langley's experiment in cats in which the sympathetic nerves had degenerated and still obtained dilatation

Knowing the importance of vasomotor reactions to the efficient working of the normal body and having established the powerful influence of the autonomic nervous system on these reactions in all parts of the vascular system, it is important to find out whether variations in them occur in certain pathological conditions in which the flow in the vessels is abnormal   A fruitful field of investigation seemed to be the study of the vascular reactions of the capillaries during passive congestion   The abnormality of the blood flow in the capillaries in cases of advanced heart disease with chronic passive congestion and cyanosis has been described frequently   It has seemed to the author that these patients as compared to normal individuals showed a delayed and even diminished response to various stimuli such as the sudden change from a hot to a cold temperature or vice versa, fright,

etc. If this is so its importance to the organism must be very great
In order to see whether this was so the effect of stimulation of the
cervical sympathetic on the capillaries of the ear during passive con-
gestion has been compared to the normal reaction

Albino rabbits of 1 5 to 2 kilos were used for the experiments
Ether was used as the anesthetic. At first considerable difficulty was
experienced in obtaining a satisfactory production of congestion
Compression of the veins in the neck proved unsatisfactory as the
collateral circulation was too free Compression of the base of the
ear would have sufficed but by this means a reduction of the arterial
supply would have been obtained also Finally the problem was
solved by dissecting out the main ear veins and producing the requisite
amount of traction on them by means of loops of thread passed under
the veins The sympathetic nerve was exposed on the side corre-
sponding to the ear used and was stimulated by faradic current The
ear was fixed flat on the stage of a Leitz microscope and mineral oil
was applied to its surface The magnification used to study the
capillaries was 40 times Light was provided by a 5 ampere direct
current arc lamp It passed through a condenser and then a filter
which contained a solution composed of a mixture of copper sulphate
and potassium dichromate This solution was green in color so that
besides removing the heat rays it provided a better contrast between
the capillaries and the tissues than was given when ordinary light
was used The light was reflected from the mirror of the microscope
through the rabbit's ear By means of this a very satisfactory view
of the small vessels in the ear was obtained

When the preparation had been set up the capillaries were watched
under the microscope while an assistant stimulated the sympathetic
The efficacy of the latter was controlled by observation of the pupil
The time during which stimulation was applied was measured by a
stop-watch At first the changes were studied under normal condi
tions, then congestion was produced for a period varying from fifteen
to twenty minutes and the sympathetic again stimulated Finally
observations were repeated from time to time after the venous obstruc-
tion had been removed As far as possible the same capillaries were
observed during each period

Four experiments of this nature were performed and all gave the

same result   In the normal state the effect was to produce after a short latent period a marked pallor due to contraction of the arterioles When this occurred the corpuscles in the capillaries became clumped together and the flow stopped   After a short but definite interval the corpuscles again began to move toward the vein and were finally expelled so that the capillary disappeared from view   During this latter period no fresh corpuscles were seen to enter the capillary from the arteriole   There was no doubt that the two changes described were separate processes   The latent period after which these changes took place was practically constant in repeated observations   When venous congestion was produced the ear became definitely cyanotic The capillaries were dilated and their flow markedly altered   All gradations were seen between complete stasis and those in which there was little change from normal   The rate of flow was much reduced in the majority and was comparable to the condition which had been observed in advanced cases of heart disease (11) (12)

The flow in the different capillaries varied from time to time as had been seen also in heart cases   The capillaries particularly studied were those in which the rate of flow was considerably reduced   In these no contraction was obtained from stimulation even when it was continued for five or six times as long as had been necessary to cause disappearance of the capillaries under normal conditions   Some capillaries were observed in which the flow was only moderately reduced and in these contraction took place after a prolonged latent period In those in which the flow was almost normal the latent period was only slightly increased   On removing the venous obstruction it was found that gradual recovery took place   Capillaries which had failed to contract during the period of obstruction began to contract after a long latent period   The latent period became less and less until finally it reached the normal level

The following is the protocol of a typical experiment

Albino rabbit   Weight 1 5 kilos   Ether anesthesia   Main ear veins in right ear dissected out and loose threads passed round them   Right cervical sympathetic exposed in the neck for stimulation   Right ear placed on the stage of the microscope and examined

Time	Duration of stimulation	Rate of blood flow	Result
	seconds		
10 31	7	Normal	Contraction
10 33	8	Normal	Contraction
10 35	7	Normal	Contraction
Venous obstruction produced 10 37			
10 40	13	Capillary observed showed only slight slowing	Contraction
10 42	24	Marked slowing	No contraction
10 46	32	Marked slowing	No contraction
10 51	28	Marked slowing	No contraction
10 57	15	One vessel with slight slowing	Contraction
		Those with marked slowing	No contraction
Venous obstruction removed 10 58			
11 01	15	Slight slowing	Contraction
11 06	10	Almost normal	Contraction
11 12	6	Normal	Contraction
11 33	8	Normal	Contraction
11 36	7	Normal	Contraction

There was little doubt that the changes which took place in the capillaries were independent of those taking place in the arterioles However, it was felt that if a substance could be used which dilated the arterioles but left the capillaries unaffected this possibility could be definitely ruled out. For this purpose acetylcholine was used Taveau and Hunt (13) first showed the powerful vasodilator action of this substance in extremely dilute solution and Hunt (14) later proved that the action was independent of vasomotor nerves Dale and Richards, (15) in their classical paper in which they analyzed the action of various vasodilator substances, found that this drug acted powerfully on the arterioles but they were unable to find any evidence of an action on the capillaries They also showed that the changes produced were not dependent on vasomotor nerves Acetylcholine has the further advantage that it produces a very marked vasodilatation of the cutaneous vessels The effect of a single injection of acetylcholine passes off very rapidly so that it was necessary to give a continuous injection in order to maintain a low blood pressure during the entire period of stimulation A solution of acetylcholine

(1 cc = 0 002 mgm) was allowed to flow from a burette into the jugular vein at a rate which it had been found in experiments on other rabbits of similar weight would maintain the blood pressure at 20–30 mm Hg below normal   It would have been preferable to have had the blood pressure reading on the animal used for the experiment but the many other details which required attention and the fact that the head was used for observation rendered it advisable to eliminate this

Two experiments were performed in which the procedure was the same as in the previous experiments with the addition of the injection of acetylcholine during the period of stimulation   As a preliminary acetylcholine was injected in each instance without sympathetic stimulation in order to see whether it produced any action on the capillaries   The only effect observed was a slight slowing of the stream   In some instances it was thought that a slight dilatation took place but this was not constant and when seen was so slight that it was uncertain   The changes caused by sympathetic stimulation in these experiments differed from those described above in that during the normal period the marked pallor due to arteriolar constriction was absent or markedly reduced while the latent period before contraction of the capillaries took place was about doubled Stimulation during the period of congestion was without effect on the capillaries in which the rate of flow was greatly reduced as was found in the other experiments

DISCUSSION

The results of the present series of experiments confirm those obtained by previous authors that sympathetic stimulation causes contraction of the capillaries independent of its action on the arterioles   Not only was contraction of the capillaries produced in the normal animal but it also took place after the arterioles had been dilated by acetylcholine   They also showed that a profound modification of the action of the sympathetic nervous system on the capillaries takes place during passive congestion   In marked degrees of congestion the capillaries failed to react to sympathetic stimulation and in the lesser degrees the effect was considerably modified   A similar change has been observed by Lewis (16) in man   He found when the pressure in a cuff round the arm was raised more than from

40 to 60 mm Hg he failed to obtain blanching on puncturing adrenalin (1–1000) into the congested area

It is of considerable interest to consider the cause of the altered response during passive congestion   One possibility is that it may be due to the accumulation of acid metabolites, particularly CO_2 in the vessels and tissue spaces   This supposition was mainly suggested by perfusion experiments in which capillary dilatation was produced by acid solutions of much greater acidity than one would find under living conditions   However Krogh (17) has shown that a considerable increase of CO_2 in the circulation is without effect on the capillaries while it required the application of very acid mixtures to the frog's tongue to produce any dilatation of the capillaries   As Lewis (16) points out it is improbable that an accumulation in the vessels or tissue spaces of such volatile and weak acids could induce such prolonged effects as these substances would be rapidly removed when normal conditions were restored

The second possibility that suggests itself is that the profound changes in the capillaries were due to anoxemia   Krogh (17) showed that the changes, which took place in reactive hyperemia following circulatory arrest with complete stasis, were not prevented either in the frog's tongue when it was kept in an atmosphere of pure oxygen so that no cyanosis took place or in the frog's web which was amply supplied with oxygen from the atmosphere   Lewis (16) has demonstrated that this reaction in man takes place after such a short period of stasis as to make it most improbable that it is due to a metabolic change from anoxemia

The third possibility is that it is due to the accumulation in the tissue spaces during the period of stasis of some substance which acts on the capillaries   Lewis (16) has brought forward strong evidence to show that a non volatile, slowly diffusible substance is constantly produced by the tissues and that it can produce a profound effect on the capillaries when allowed to accumulate   This he terms "H substance" but he shows that it is probably histamine as it resembles the latter so closely in its effects   He has also demonstrated that histamine is capable of preventing or abolishing the response of the vessels to adrenalin   This explanation seems to be the most likely for the changes observed in the present experiments   Obstruc-

tion of the venous flow would permit an accumulation of the dilator substance while the gradual recovery observed would be compatible with the removal of a slowly diffusible product such as the "H-substance"

Although not directly connected with the subject under investigation it was seen that the latent period following sympathetic stimulation before the capillaries contracted was increased when acetylcholine was injected The changes observed in the capillaries following the injection of acetylcholine when no stimulation of the sympathetic was used were very slight and confirmed the view that the arterioles were mainly responsible for the fall in blood pressure They did not suggest that the prolonged latent period was directly due to a change in the capillaries Under normal conditions the arterioles contract after stimulation of the sympathetic and thus prevent fresh blood from entering the capillaries while after acetylcholine the arterioles remain dilated and allow blood to flow freely into the capillaries Under these circumstances the capillaries would have a greater force to contract against than when a few motionless corpuscles lie in their lumen Thus the time required for their complete disappearance would become lengthened

CONCLUSIONS

1 Peripheral stimulation of the cervical sympathetic caused the capillaries in the rabbit's ear to contract This effect took place at a definite interval after arteriolar contraction It was also present when the arterioles were dilated by acetylcholine

2 The latent period between the commencement of stimulation and contraction of the capillaries was prolonged when acetylcholine was injected throughout the time of stimulation

3 The production of congestion by venous obstruction caused either failure of the capillaries to contract as a result of stimulation of the sympathetic nervous system or a considerable prolongation of the latent period depending on the degree of stasis in the individual capillaries

4 It is suggested that individuals with chronic passive congestion show a variation from the normal in their vasomotor reactions, dependent on the degree of stasis

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THE EXCRETION OF ZINC IN HEALTH AND DISEASE

By LAWRENCE T FAIRHALL and LYMAN H HOYT

(From the Department of Physiology Harvard School of Public Health and the Medical Clinic Peter Bent Brigham Hospital Boston)

During recent years increasing attention has been directed towards the occurrence of zinc as a normal constituent of animal tissues and fluids (1) Its wide distribution in foodstuffs and particularly its concentration in germ cells—the endosperm of wheat and the yolk of eggs, for example—and constant presence in milk points to a definite utilization The fact that there is a more or less definite ratio of zinc to calcium in blood in the proportion of 7 100, and that zinc metabolism in animals has been shown to bear some relation to inorganic salt metabolism in general (2), leads to speculation as to the part it plays in the human organism

For this reason it was felt desirable to secure further data regarding the excretion of absorbed zinc under conditions of disease as compared with the normal It is interesting to note that man ingests in his food practically as much zinc as he does iron Far the greater part is excreted through the intestine A small amount, however, is absorbed and excreted through the urine and, as the latter is a better index of absorbed zinc, a series of analyses of urinary zinc under conditions of health and disease was carried out

EXPERIMENTAL

Twenty four hour specimens of urine were collected from a number of healthy individuals and also from hospital patients suffering from various diseases such as nephritis, uremia, duodenal ulcer, tuberculosis and diabetes In order to avoid incidental contamination with zinc from glassware, the urine was collected in Pyrex glass-stoppered bottles and evaporated to dryness and ashed in Pyrex dishes The zinc was separated and determined by a turbidimetric method as the ferrocyanide (3)

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Various clinical pathological data were collected also for comparison with the zinc data   Blood studies included blood urea nitrogen determinations, red blood cell counts, white cell counts and hemo-globins   The presence or absence of edema was noted   Blood pressures and the degree of existing vascular disease were also recorded

TABLE 1

Normal urinary excretion of zinc in health

Subject	Urine volume (24 hours)	Total zinc per day	Zinc per liter per day
	cc	*mgm*	*mgm*
1	1,200	0 48	0 40
2	1,487	0 69	0 46
3	1,300	0 41 *	0 31
4	1,610	0 41	0 26
5	1,320	0 28	0 21
6	895	1 00	1 12
7	1,075	0 27	0 25
8	2,260	0 41	0 18
9	1,022	0 59	0 58
10	1,312	0 90	0 68
11	1,140	0 69	0 60
12	795	1 12	1 41
13	975	0 91	0 93
14	626	0 27	0 43
15	1,390	0 27	0 19
16	1,022	1 18	1 15
17	540	0 69	1 28
18	2,092	1 12	0 54
19	1,011	0 34	0 34
20	1,724	1 00	0 58
21	310	0 27	0 87
22	1,575	0 80	0 51
23	836	0 60	0 72
24	1,340	0 45	0 34
Average	1,202	0 64	0 60

Urine examinations for albumin and casts were made   Owing to the fact that none of these figures show any significant correlation with the zinc output they have not been included in the tabulated data The normal zinc content of the urine of the twenty-four healthy individuals is shown in table 1, while the zinc content of the urine of the patients studied is shown in table 2

TABLE 2
Urinary excretion of zinc in disease

Subject	Type of disease	Urine volume	Total zinc per day	Zinc per liter per day
		cc	*mgm.*	*mgm.*
25	Nephritis	310	0 17	0 55
26	Nephritis	305	0 75	2 45
27	Nephritis	321	0 40	1 25
28	Nephritis	1,836	1 30	0 71
29	Nephritis	516	0 70	1 36
30	Nephritis	1,109	0 70	0 63
31	Nephritis	588	0 25	0 43
32	Nephritis	432	0 55	1 27
33	Nephritis	205	0 65	3 17
34	Nephritis	575	0 55	0 96
35	Nephritis	298	0 70	2 35
36	Nephritis	437	0 65	1 49
37	Nephritis	148	0 87	5 88
38	Nephritis	2,337	0 50	0 21
39	Nephritis	880	0 60	0 68
40	Nephrosis	558	0 54	0 97
Average		678	0 61	1 52
41	Uremia	1,540	1 65	1 07
42	Uremia	760	0 87	1 15
43	Uremia	1,310	1 45	1 10
Average			1 32	1 11
44	Duodenal ulcer	1,720	1 46	0 85
45	Duodenal ulcer	1,010	0 38	0 38
46	Duodenal ulcer	1,580	1 40	0 91
47	Duodenal ulcer	1 510	0 65	0 43
48	Duodenal ulcer	1,060	0 15	0 14
49	Duodenal ulcer	950	0 45	0 47
50	Duodenal ulcer	550	0 35	0 64
51	Duodenal ulcer	1 750	0 45	0 26
52	Duodenal ulcer	2,250	0 87	0 39
53	Duodenal ulcer	835	1 05	1 26
54	Duodenal ulcer	2 175	0 60	0 27
Average			0 71	0 54
55	Tuberculosis	1 985	1 55	0 78
56	Tuberculosis	850	0 70	0 82
57	Tuberculosis	760	1 08	1 42
58	Tuberculosis	1,000	1 24	1 24
Average.			1 14	1 06

TABLE 2—*Concluded*

Subject	Type of disease	Urine volume	Total zinc per day	Zinc per liter per day
		cc	*mgm*	*mgm*
59	Pernicious anemia	1,490	1 03	0 69
60	Pernicious anemia	450	0 55	1 22
61	Lymphatic leukemia	800	0 76	0 95
62	Cardiac	978	0 27	0 28
63	Diabetes	850	0 70	0 82
64	Gout	1,190	1 35	1 14
65	Myxedema	305	0 65	2 13

DISCUSSION

None of the clinical pathological figures obtained from the urea nitrogen determinations, complete blood counts, blood pressure measurements, nor the presence or absence of edema, vascular disease, nor urinary findings were of significance with respect to the zinc output Nor was the output of zinc greatly altered in cases of disease as compared with the normal amount excreted if the basis of comparison is *total output* In the case of nephritic patients, the zinc concentration is increased above that of the normal because the total urinary output was small Whereas the average normal daily output was 0 64 mgm per 24 hours, or 0 60 mgm per liter, with nephritic patients the average total daily output was 0 61 mgm per 24 hours, or 1 52 mgm per liter Although this is a much higher concentration than normal, it should be noted that the total daily urinary output of the patients with nephritis studied represented but slightly over one-half that of the normal individuals This was the direct result of the constant restricted low fluid intake which was part of the treatment If the urinary volume output of the nephritic patients had not been restricted in this manner, there is the possibility that the total zinc excretion figure would have been affected, that is, if the average urine volume of the nephritic patients had been as great as the normal, it is a reasonable assumption that the total zinc output might have been increased in proportion In any event, in terms of concentration alone, the urinary zinc of the nephritic patients was greater than that of the normal individuals

Patients with duodenal ulcer show no great departure from the

average for normal individuals   The average total daily excretion of the ulcer cases was 0 71 or 0.54 mgm per liter per day

In uremia and tuberculosis, the zinc content was distinctly higher than the average normal in every case examined   The average daily excretion of tuberculous patients was 1 14 or 1 06 mgm per liter, while in uremia, the average daily excretion was 1 32 or 1 11 mgm per liter In these last two cases, therefore, the average zinc excretion was practically double the average normal excretion   Whether these differences are great enough to be of significance is a debatable question   With this as a clue, however, it might prove of interest to investigate the zinc metabolism of tuberculous patients more closely

SUMMARY

An investigation of the urinary zinc excretion of twenty-four healthy individuals and of forty-one hospital patients showed that while no constant relation could be discovered between zinc output and the degree of anemia, or alteration in hemoglobin, blood cell count, urinary albumin or edema, the zinc output is somewhat different in nephritis and markedly accentuated in uremia and tuberculosis

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SKIN REACTIONS TO FILTRATES OF HAEMOLYTIC STREPTOCOCCI IN ACUTE AND SUBACUTE NEPHRITIS[1]

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In a previous communication (1) it was noted that haemolytic streptococci isolated from the tonsils, adenoids or accessory nasal sinuses of cases of acute and subacute glomerular nephritis produced, in broth cultures, "toxins" which caused skin reactions similar to those caused by the "Dick Toxin." While studying the property of these filtrates, it was observed that intense skin reactions often occurred in patients suffering from acute and subacute nephritis associated with infections caused by haemolytic streptococci A systematic study was, therefore, made of the incidence and intensity of the skin reactions to these bouillon filtrates of haemolytic streptococci in three groups of cases, first, patients suffering from acute and subacute nephritis, secondly, normal individuals and patients affected with miscellaneous conditions, and thirdly, patients suffering from uncomplicated acute tonsillitis There will be no attempt to discuss at this time the nature of these filtrates

It is, however, to be noted that many observers have found that haemolytic streptococci recovered from various forms of infection are capable of elaborating "toxic" substances when grown in broth (2, 3, 4, 5, 6, 7, 8, 9, 10, 11)

Although these filtrates are usually more resistant to heat and less potent than the "Dick Toxin," yet they possess other properties in common with the "Dick Toxin," for they are frequently neutralizable by antiscarlatinal serum (3, 7, 9, 10) and are considered by some to differ only quantitatively from the "Dick Toxin," though qualitative differences have also been observed (10, 11, 13, 13a)

The significance of the Dick reaction and of the reactions caused by filtrates obtained from the growth of non-scarlatinal strains of haemolytic streptococci is not perfectly clear   Mackenzie and Hanger (5) considered that the reactions which they obtained indicated an acquired allergy, and that the skin reaction was probably similar in nature to the local tuberculin reaction   There is increasing evidence to sustain this point of view   Dochez and Sherman (12) have shown that the skin of rabbits and guinea pigs, previously inoculated with living cultures of *Streptococcus scarlatinae*, becomes sensitive to filtrates of this organism, while Zinsser and Grinnell (13) have observed that the pronounced skin reactions to haemolytic streptococcus filtrates and to the "Dick Toxin," which can be obtained in guinea pigs infected with haemolytic streptococci, may fade or disappear after the infection has persisted for some days, and later reappear again   They conclude that these reactions are allergic in nature and comparable to the tuberculin reaction   We have observed the same phenomena in rabbits infected subcutaneously by strains of haemolytic straptococci obtained from infections in acute nephritis The skin of the rabbit which, under normal conditions gives no reaction to the intracutaneous injection of 0 2 cc of undiluted filtrate, may react to the same filtrates within five to seven days after the onset of an induced local streptococcus infection, by an area of erythema and oedema 1–2 cm in diameter   Two or three weeks later the hypersensitiveness of the skin usually disappears   We could not determine that these reactions were specific for different strains Infection of the rabbit by one strain of haemolytic streptococcus rendered the skin sensitive to filtrates from many strains of haemolytic streptococci as well as to the "Dick Toxin"   Dochez and Stevens (14) have shown that two phases of cutaneous allergy develop when rabbits are immunized with filtrates of haemolytic streptococci from erysipelas   The reactions occurring during the first period of allergy can be neutralized with erysipelas immune serum, but the reaction during the second phase cannot

The important observations of Cooke (15), upon the nature of the Dick reaction, bring strong evidence to show that the Dick reaction depends upon an allergy acquired, through streptococcus infections, during early life   A positive reaction may become temporarily

negative through desensitization or be rendered permanently nega-
tive by the production in the body of antitoxin   Kirchner (16)
states that he can sensitize the cornea of rabbits against filtrates
of both scarlatinal strains and non scarlatinal strains of haemolytic
streptococci

METHODS

In the present investigation 18 strains of hemolytic streptococci were employed
Thirteen of them were of the beta type and five of alpha type. The organisms
were grown in beef infusion peptone broth of pH 7 4 for 18 hours at 36 5°C,
which was then filtered through Berkefelt candles and Seitz filters   For skin
reactions the filtrates were usually employed in 1–100 dilutions   When positive
skin reactions were obtained with these dilutions, tests were made as a rule with
1–500 1–1000, 1–2000 and occasionally 1–5000 dilutions   For each test 0 1 cc.
of the dilution was employed   Stock bouillon in 1–100 dilution was employed as a
control and a Dick reaction with 1 S T D of toxin" was always made at the same
time   The skin reactions were read at the end of 18 hours and 24 hours   The
reaction was considered negative unless the area of erythema measured more than
1 0 cm in one diameter   Reactions of 1 to 1 5 cm were termed +, reactions of
1 5 to 2 0 cm were termed + + reactions of 2 0 to 2 5 cm in diameter were termed
+ + + and all reactions 2 5 cm in diameter or over were designated + + + +

Since the observations have been carried on over several years it has been neces-
sary to prepare many batches of filtrates   These have been titrated against a
known reactor before they were employed for tests   It was surprising to note with
what regularity a single strain produced a filtrate of approximately the same
strength

Skin reactions in normal persons and patients suffering from
miscellaneous conditions

Mackenzie and Hanger (5) found young children quite regularly
insensitive to the filtrates which they employed, but obtained positive
reactions in a very large proportion of adults   They were not able to
correlate these reactions with previous or existing infections, nor
could they find any apparent relation between the presence of these
skin reactions and any one disease or group of diseases   They
employed comparatively large doses of filtrate using 0 03 to 0 04 cc
of undiluted broth filtrates   Howell and Corrigan (17) found that a
high percentage of persons, both healthy and diseased, reacted posi-
tively to streptococcus filtrates, and that there was no apparent cor-
relation between the skin reactions and the etiological factors of the

disease Children that were susceptible to streptococcus infections gave a higher percentage of positive reactions with all streptococcus filtrates than did other children or adults They suggest that a positive skin reaction with streptococcus filtrates may indicate susceptibility to streptococcus infections Lash and Kaplan (2) obtained 8 1 per cent positive skin reactions in 247 women who were either normal (20), pregnant (86), or puerperal (141), with filtrates in 1–1000 dilution of a streptococcus obtained from a case of puerperal septicaemia Birkhaug (6) found that 21 per cent of school children, between the ages of 7 and 17 years, gave positive skin reactions to the toxin from *Streptococcus erysipelatis*, while 27 per cent of 135 hospital patients, between the ages of 17 and 72 years, gave positive reactions to 1 S T D

Amoss, Hansen-Pruss and Bliss (18) state that 70 per cent of normal men, and 80 per cent of normal women give more or less marked skin reactions to erysipelas toxin in 1–100 dilution

Statistics concerning the occurrence of positive Dick reactions in children and adults vary considerably Dick and Dick (19) give 58 8 per cent of positive reactions in 1250 persons, Lees (20) 49 8 per cent positive reactions amongst 530 University students, Zingher (21) 26 3 per cent positive reactions amongst children in New York City while Dyer (22) found that school children from rural and urban districts might show a variation in positive reactions from 100 per cent to 25 per cent

It is obviously impossible to make accurate comparisons of the incidence of positive skin reactions to streptococcus filtrates obtained by different observers So much depends upon the dosage, the strength of the various filtrates and the group of individuals tested It is possible, however, to compare with a considerable degree of accuracy the tests performed with standardized filtrates by a single observer in different groups of patients

We have tested the skin of 60 individuals, who were supposedly normal or who were suffering from a miscellany of conditions, with bouillon filtrates of the strains of haemolytic streptococci isolated from infections in acute nephritis Eighteen different strains were employed in the preparation of these filtrates, and the filtrates from 2 to 16 strains were employed for tests on each individual An average

of 9 filtrates was used for tests in each person   Most of these persons
were between 18 and 35 years of age   Of the 60 individuals one half
were supposedly normal, the other half, though not acutely ill, were
affected with a variety of conditions, such as chronic valvular heart
disease, duodenal ulcer, multiple sclerosis, hyperthyroidism, chronic
tonsillitis, diabetes, hereditary telangiectasis   chronic sinusitis,

TABLE 1
Incidence of reactions   Normals

	Total number	+ or more		++++		Dick +	
		Number	Per cent	Number	Per cent	Number	Per cent
Subjects	60	38	62 2	15	25	14	20 3
Tests	542	167	30 8	33	6		

TABLE 2
Reactions with less dilute filtrates   Normals

Subject	Strain of filtrate	Intensity of reaction at following dilutions of filtrate		
		1 100	1-50	1-20
1	a	0	+++	
	b	++	++++	
2	a		0	0
	b		0	0
3	a	0	0	
	b	+	++++	
	c	0	+++	
4	a	0	+	++
	b	0	0	+
	c	0	0	
	d	+	+	+++

arteriosclerosis, chronic tuberculosis, convalescence from pneumonia
and typhoid fever
    Table 1 gives the incidences of total reactions, the incidence of
strong reactions and the proportions of positive reactions in this group
of control cases
    It was found that about two thirds of all these patients gave some

reaction to one or more of the filtrates in 1–100 dilutions, but that only one-fourth of the patients gave strong reactions in this dilution Comparatively few patients gave reactions to dilutions of 1–500 or more   Of 33 individuals, selected largely from those who had reacted to the filtrates in 1–100 dilutions, who were tested with a 1–500 dilution of the filtrates 15 or 45 4 per cent gave + or greater reactions, while only 3 or 9 per cent gave + + + + reactions

Four of the entire number of the negative reactors were tested with less dilute filtrates, 1–50 and 1–20 being employed   Two individuals who gave negative reactions with 1–100 dilutions showed + + + reactions with 1–50 dilutions   One individual gave a negative reaction at 1–50 but a + reaction at 1–20, and one individual gave negative reactions both with 1–50 and 1–20 dilutions   The titrations of these reactions are given in Table 2

The positive Dick reactions were obtained almost entirely among the individuals that gave positive reactions to the bouillon filtrates Of the 14 positive Dick reactions 13 occurred amongst the positive reactors and one amongst the negative reactors

It is important to draw attention to the fact, that, in the miscellaneous group, there were five individuals who suffered from chronic sinusitis or chronic tonsillitis   All five of these patients gave positive reactions and four of them showed + + + + reactions to one or more of the filtrates   Furthermore all of these five individuals came in the group of 15 who gave + reactions in 1–500 dilutions, while the 3 individuals who gave + + + + reactions in 1–500 dilutions belong to this group of chronic tonsillitis and sinusitis

From the observations made on these 60 persons, it may be concluded that the skin reactions to these bouillon filtrates of haemolytic streptococci vary considerably in different individuals   The skin of some individuals may be almost insensitive to comparatively large doses of the filtrates, while the skin of other persons may react quite vigorously to considerably diluted filtrate   It was also observed that reactions to the same filtrates might vary from time to time   In several individuals reactions that were at first + + to + + + + became on subsequent tests +   It was also noted that in this control group the individuals having obvious chronic tonsillitis or sinusitis were among those most likely to give strong reactions

Skin reactions in acute and subacute nephritis

The results obtained from the skin tests made upon patients suffer
ing with acute and subacute nephritis differed, in several respects, from
those in the control groups

Twenty-seven patients were tested   An average of 9 filtrates from
different strains was used in each patient   Table 3 shows the number
and percentage of positive reactions obtained in these twenty seven
patients with 0 1 cc of 1–100 dilutions of filtrates

It may be seen from the table that this group of patients suffering
from nephritis gave reactions much more frequently than the control
groups, that they gave many more $++++$ reactions, and that they
reacted to a greater number of filtrates   When one measures the
susceptibility of these patients to the streptococcus filtrates, by titrat-

TABLE 3
Incidence of reactions   Nephritis

	Total number	+ or more		$++++$		Dick +	
		Number	Per cent	Number	Per cent	Number	Per cent
Patients	27	22	81 4	18	66 6	9	33 3
Tests	259	162	62 5	62	23 9		

ing the dose of filtrate, the difference becomes even more striking   Of
12 patients tested with 1–500 dilutions 9, or 75 per cent gave $+$ to
$++++$ reactions, whereas in the control group only 45 4 per cent of
33 cases tested with 1–500 dilutions gave $+$ reactions   Of the 12
nephritics 6, or 50 per cent, gave $++++$ reactions with 1–500
dilutions whereas only 9 per cent of the controls gave $++++$
reactions with this dilution   In the cases of nephritis, many positive
reactions were obtained with still greater dilution, of filtrate   Of
7 patients tested with 1–1000 dilution, 5 gave $+$ reactions or greater
and 2 $++++$ reactions   Of 5 patients tested with 1–2000 dilutions,
2 gave $++++$ reactions, and one of two patients tested with 1–5000
dilutions gave a $+$ reaction

In the group of nephritics, there seemed to be no definite relationship
between reactions from filtrates and reactions from "Dick Toxin"
There were 9 of the 27 patients who gave positive Dick reactions   All

of these gave ++++ reactions with the bouillon filtrates Of the 18 patients who gave negative Dick reactions 9 or 50 per cent gave ++++ reactions with the bouillon filtrates It can only be said, therefore, that when the Dick reaction was positive the reactions with filtrate were strongly positive, but that strongly positive reactions with filtrates occurred in one-half the patients that gave negative Dick reactions

Amongst the 27 nephritics, there were 4 cases that gave no reactions to any filtrates of the streptococci, and that also gave negative Dick reactions Three of these patients had marked oedema at the time the tests were made One of these three, who had much oedema at the time the first tests were made, was retested later after the oedema had subsided and still showed completely negative reactions The fourth had only moderate oedema It is possible that marked oedema of the subcutaneous tissues may favor rapid absorbtion of the injected bacterial filtrate, such as occurs with sodium chloride solution, and thereby reduce the liability of a reaction in the skin, though it is more probable that the skin of these patients was actually insensitive to the filtrates

The skin reactions in this group of 27 nephritics seemed to persist in the same degree and intensity in many patients for months or even years Two patients have shown skin reactions of the same intensity for 8 months, 2 for 1 year, 1 for 1 year and 8 months, one for 2 years, 2 for 2 years and 8 months, and one for 3 years Among this group of 9 retested patients, there are three who have, apparently, recovered completely from their attacks of acute nephritis as well as from the infection The acute nephritis in each instance was associated with an acute tonsillitis due to β haemolytic streptococci In all three, tonsillectomy was performed during the early stage of nephritis Repeated examinations over a period of 8 months in one case and 2 years and 8 months in the other two have shown that they were free from infection, and cultures from the pharynx and nasopharynx have not shown β haemolytic streptococci Three of the 9 patients retested are symptomatically well, but still show traces of albumen and occasional hyaline casts in the urine, while cultures from the pharynx have shown from time to time haemolytic streptococci, in the remaining group of three of the total 9 cases, the original acute or subacute

nephritis has become chronic, infections have persisted and cultures from the infections of the pharynx and paranasal sinuses have shown constantly haemolytic streptococci

These observations indicate that the increased sensitiveness of the skin of patients, suffering from acute and subacute nephritis, may persist for long periods of time, and that skin reactions of great intensity, in these patients, are not necessarily dependent upon the existence of a demonstrable infection or upon the carrier state

It seemed necessary, however, to obtain further information as to the relation of the positive skin reaction to acute infections, produced by haemolytic streptococci of β type, occurring in patients in whom there was no evidence of a complicating acute nephritis   For this purpose, cases of uncomplicated acute tonsillitis were selected   Skin

TABLE 4

Incidence of reactions   Tonsillitis

	Total number	+ or more		++++		Dick +	
		Number	Per cent	Number	Per cent	Number	Per cent
Subjects	22	21	95 4	4	18 1	11	50
Tests	123	70	56 9	4	3 2		

reactions were performed with the bouillon filtrates in 22 such cases Haemolytic streptococci were obtained in cultures from the tonsils in all cases   An average of five filtrates was employed for the tests in each case.   In all but four, the filtrates were used in 1–500 dilutions, and therefore the results are not exactly comparable to those obtained in the control groups   The table 4, however, shows the incidence of positive reactions in this group of uncomplicated tonsillitis

It will be seen that though a very large percentage of these cases gave positive reactions, a comparatively small number showed strongly positive reactions in 1–500 dilution, in the tonsillitis group 18 1 per cent and in the nephritis group 50 per cent.   Though the total number of cases is small, the pronounced variations are indicative of considerable difference in the sensitiveness of the three groups to the bouillon filtrates

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the preponderance of strongly positive skin reactions in the nephritic group, for the nephritics continued to show this peculiarity, in several instances, for months or years after the streptococcus infection had disappeared Moreover, if one regards the results from another point of view, it appears that a fair proportion of all the individuals who gave strongly positive skin reactions were nephritic. Of the total number of 109 individuals tested, 37 or 33 9 per cent gave strongly positive reactions, and of these 37 strongly positive reactors, 18 or 48 6 per cent were nephritic

There appears, actually, to be a quantitative difference in the skin reactions to these filtrates, between many patients with nephritis and the average normal individual, or the patient suffering from an uncomplicated acute streptococcus tonsillitis This difference consists in a heightened susceptibility of the skin to the culture filtrates of haemolytic streptococci It seems possible, that there may be some connection between the heightened susceptibility of the skin to these filtrates, and the occurrence of acute glomerular nephritis as a complication of local streptococcus infection It is becoming more and more obvious that in the streptococcus infections, as in tuberculosis and syphilis, an induced allergy, towards the protein of the streptococcus or to the products of its growth, accounts for some of the variations in the form of the infection and of its complications Bristol (23) has expressed the view that the exanthem of scarlet fever is an allergic reaction to the local streptococcus infection, and has reviewed the literature on this subject The more recent investigations by Dochez and Stevens (14) and by Cooke (15) go far to substantiate this idea Dochez and Stevens conclude that both the Dick reaction and the rash in scarlet fever are dependent upon previous sensitization of the individual to haemolytic streptococci, and are to be looked upon as allergic reactions of the individual to products of *Streptococcus scarlatinae* Cooke adds considerable evidence to confirm this hypothesis in which he thoroughly concurs

Another streptococcus infection, which presents certain features that have been interpreted as allergic in nature, is erysipelas Amoss, Hansen Prüss and Bliss (18) bring evidence to show that recurrent attacks of erysipelas are allergic in character, and find that the skin reactions to bouillon filtrates of erysipelas strains of haemolytic

DISCUSSION

A study of the skin tests made with filtrates of broth cultures of haemolytic streptococci, in these three groups of cases, shows the necessity, in any comparative investigation, of using different dilutions of the filtrate to distinguish varying degrees of reactivity of the skin Amongst the individuals in the three groups, one finds, by titrating the reaction, all transitions from those, who are practically insensitive to the filtrates in the doses employed, to those who react vigorously to 1–2000 dilutions The control group includes the largest proportion of poor reactors, whereas the nephritic group contains the largest number of strong reactors The tonsillitis group contains many reactors, but comparatively few strong reactors Table 5 demonstrates quite clearly these differences

TABLE 5

Summary of incidence of reactions

Diagnosis		Total number	+		++++	
			Number	Per cent	Number	Per cent
Cases	Controls	60	38	62 2	15	25
	Tonsillitis	22	21	95 4	4	18 1
	Nephritis	27	22	81 4	18	66 6
Tests	Normal	542	167	30 8	33	6 0
	Tonsillitis	123	70	56 9	4	3 2
	Nephritis	259	162	62 5	62	23 9

In the nephritic group, not only was the proportion of strongly positive reactions very high, but each positive reactor in the group showed strongly positive reactions to many more strains than the positive reactors in the other groups Though the total number of cases is not large, yet the differences are so pronounced between the groups, that one is inclined to attribute them to actual variations in the susceptibility to the filtrates rather than to chance The results obtained, both in the control group and in the tonsillitis group, indicate that persons suffering from various forms of infection due, to β haemolytic streptococci, are somewhat more likely to give positive skin reactions to these streptococcus filtrates than are normal individuals It does not seem, however, that this propensity can account entirely for

the preponderance of strongly positive skin reactions in the nephritic group, for the nephritics continued to show this peculiarity, in several instances, for months or years after the streptococcus infection had disappeared   Moreover, if one regards the results from another point of view, it appears that a fair proportion of all the individuals who gave strongly positive skin reactions were nephritic   Of the total number of 109 individuals tested, 37 or 33 9 per cent gave strongly positive reactions, and of these 37 strongly positive reactors, 18 or 48 6 per cent were nephritic

There appears, actually, to be a quantitative difference in the skin reactions to these filtrates, between many patients with nephritis and the average normal individual, or the patient suffering from an uncomplicated acute streptococcus tonsillitis   This difference consists in a heightened susceptibility of the skin to the culture filtrates of haemolytic streptococci   It seems possible, that there may be some connection between the heightened susceptibility of the skin to these filtrates, and the occurrence of acute glomerular nephritis as a complication of local streptococcus infection   It is becoming more and more obvious that in the streptococcus infections, as in tuberculosis and syphilis, an induced allergy, towards the protein of the streptococcus or to the products of its growth, accounts for some of the variations in the form of the infection and of its complications   Bristol (23) has expressed the view that the exanthem of scarlet fever is an allergic reaction to the local streptococcus infection, and has reviewed the literature on this subject   The more recent investigations by Dochez and Stevens (14) and by Cooke (15) go far to substantiate this idea.   Dochez and Stevens conclude that both the Dick reaction and the rash in scarlet fever are dependent upon previous sensitization of the individual to haemolytic streptococci, and are to be looked upon as allergic reactions of the individual to products of *Streptococcus scarlatinae*   Cooke adds considerable evidence to confirm this hypothesis in which he thoroughly concurs

Another streptococcus infection, which presents certain features that have been interpreted as allergic in nature, is erysipelas   Amoss, Hansen Prüss and Bliss (18) bring evidence to show that recurrent attacks of erysipelas are allergic in character, and find that the skin reactions to bouillon filtrates of erysipelas strains of haemolytic

streptococci are pronounced in all patients suffering from recurrent attacks of erysipelas Birkhaug (24) has expressed a similar view, while Francis (25) concludes, from a study of erysipelas, that allergy plays an important rôle both in the pathogenesis of the disease and in recovery from the local lesion

Some observations have likewise been made upon the allergic reactions to non-haemolytic streptococci The work of Birkhaug (26), Kaiser (27) and Swift, Wilson and Todd (28) indicates that skin reactions to various strains of non-haemolytic streptococci, or to their filtrates, are more frequent in rheumatic fever than in non-rheumatic patients, while Swift, Wilson and Todd observed a larger proportion of positive skin reactions, during the active stage of the disease, than during the latent period Swift, Hitchcock and Derick (29), moreover, have obtained general tuberculin like reactions, occasionally with reactivation of quiescent rheumatic foci, in patients with rheumatic fever who have been injected intravenously with heated killed vaccines of both green and haemolytic streptococci, or with nucleo protein of haemolytic streptococci Swift (30), in particular, has done much to further the conception that the arthritis of rheumatic fever is an allergic reaction, the result of preliminary sensitization of the joints to non-haemolytic streptococci, and Zinsser (31) is inclined to accept this hypothesis

The idea that the inception of acute glomerular nephritis may be dependent upon an allergic reaction of the kidneys is not new, for both Schick (32) and von Pirquet (33) suggested, many years ago, that the acute nephritis of scarlet fever might be interpreted as an allergic manifestation of this disease An attempt was made by one of us, some time ago, to produce nephritis in animals by repeated anaphylactic shock, (34) and though these experiments, performed with soluble proteins, did result in degenerative lesions in the epithelium of the kidney tubules, with inflammatory reactions occasionally affecting the glomeruli, the pathological picture of diffuse glomerular nephritis was not obtained, and the results do not seem applicable to an explanation of the origin of nephritis in human beings It seems possible, however, that when the body has become sensitized to an infection, the kidney may respond, in somewhat the same manner as the skin, when the products of bacterial growth are brought in direct contact with the

kidney cells   Long and Finner (35) have recently described the typical lesions of diffuse glomerular nephritis produced, in the tuberculous pig, by the injection of tuberculin into the renal artery

If the heightened skin reaction to the filtrates of haemolytic streptococci can be interpreted as allergic reactions, then our observations indicate, that many patients, suffering from acute and subacute nephritis, are quite highly allergic to some constituent of haemolytic streptococci or to substances elaborated by these organisms   Continued investigations upon the incidence of haemolytic streptococcus infection, at the onset, during the course and with exacerbations of acute and subacute glomerular nephritis, have confirmed us in the view that this relationship is too frequent and too close to be purely incidental   Repeated cultures from the urine, even during the early stages of acute nephritis, have not shown haemolytic streptococci, except in the rare instances of what is probably a focal form of glomerular nephritis, and we have not obtained any evidence to show that the diffuse glomerular nephritis is the result of an actual infection of the kidney by bacteria   Though the idea, so frequently suggested, that the diffuse glomerular lesions are produced by the direct action of a true toxin cannot be dismissed, it seems much more probable that the reaction in the kidney may be allergic in nature, similar possibly to the reaction in the skin, and dependent upon a sensitization of the kidney cells to the haemolytic streptococci or the products of their growth

If such a condition existed, one would scarcely expect absolute correlation between the skin reaction and the kidney reaction, for in other forms of sensitization this does not occur   Most patients suffering from allergic hay fever give skin reactions to the substances causing the hay fever or asthma, but they may, in addition give skin reactions to many substances that are not concerned in the production of their asthma or hay fever   Conversely, the ingestion of a particular kind of food may cause urticaria and gastro-intestinal symptoms in patients whose skins do not react to extracts of this especial food The correlation, therefore, between the skin reaction and the somatic reaction is not absolutely constant   Should the same conditions hold for streptococcus infections, it would not be surprising to encounter patients who gave strong skin reactions to filtrates of haemolytic streptococci, and yet did not develop nephritis during the course of a

streptococci are pronounced in all patients suffering from recurrent attacks of erysipelas   Birkhaug (24) has expressed a similar view, while Francis (25) concludes, from a study of erysipelas, that allergy plays an important rôle both in the pathogenesis of the disease and in recovery from the local lesion

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streptococcus infection, and, on the other hand, observe occasional instances in which nephritis occurred, during the course of a streptococcus infection, in an individual who did not show exaggerated skin reactions to the filtrate

In the groups of patients which we have studied, however, the most intense and the most numerous skin reactions to filtrates of haemolytic streptococci have occurred in those suffering from acute and subacute nephritis   Though the nephritis occurred, in the majority of instances, in persons suffering from infections caused by haemolytic streptococci, the positive skin reactions themselves, it was found, might persist long after the disappearance of the infection, and when the patient had apparently recovered from nephritis   It was, moreover, found that almost one half of the strongly positive reactors were nephritic

SUMMARY

Skin reactions to the filtrates of haemolytic streptococci were studied in three groups of cases   (1) A control group of normal individuals and patients suffering with a variety of diseases, (2) patients with acute tonsillitis, (3) patients with acute and subacute nephritis

In the control group 62 per cent were found to give positive skin reactions, but only 25 per cent strongly positive reactions, in the tonsillitis group 95 4 per cent were found to give positive skin reactions, but only 18 1 per cent strongly positive reactions, in the nephritis group, however, 81 4 per cent gave positive skin reactions, and 66 6 per cent strongly positive reactions

The positive skin reaction is regarded as an evidence of allergy to the haemolytic streptococcus or the products of its growth

The preponderance of strongly positive reactions in the nephritis group indicates that these patients may be highly allergic to the haemolytic streptococcus or the products of its growth

It is suggested that the development of acute diffuse glomerular nephritis, in patients suffering from haemolytic streptococcus infections, may be referable to the products of the growth of the haemolytic streptococcus acting upon previously sensitized kidney cells

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THE COLOR OF THE SKIN AS ANALYZED BY SPECTROPHO-TOMETRIC METHODS

I APPARATUS AND PROCEDURES

By CHARLES SHEARD AND LOUIS A. BRUNSTING

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The color of the skin, to a certain extent, serves as an index of the state of well being, or the converse, and has been interpreted, after various modes and manners of expression, as evidence of health or disease   On the one hand there is the tanned, full-blooded skin with the texture and sheen of velvet and, on the other hand, the pale, anemic, wrinkled and lifeless skin   To be sure, the normal person exhibits a wide variation or range in the color attributes of the skin, depending on the race, habitude, occupation and exposure to the elements   Without doubt variations in environment or bodily temperature and psychic emotion or unrest play a part in the composite of factors which go to make up the color of the skin   In pathologic conditions, in which there may be abnormalities or derangements of pigmentation and of the vascular distribution (both as to quantity and quality of blood) there is a wide range of variations in the color of the skin   Some of these changes may be recognized readily by the eye, others cannot be so recognized and, if appreciated, cannot be given quantitative estimation by the clinical methods in vogue

It would seem as though greater accuracy should be introduced in clinical records regarding the color of the skin   This is particularly true in such conditions as jaundice, hemochromatosis, anemia, poly-cythemia, cyanosis, Addison's disease and in other pigmentary and vascular disturbances in which color plays a part in the diagnosis, and influences judgment regarding favorable or unfavorable progress during the course of treatment

That there has not been uniformity in correctly or adequately expressing the color of the skin is attested by numerous clinical reports

in the literature in which are used such expressions as reddish hue, bluish hue, death-like, bronzy, livid, cyanotic, and the like The language used for expressing conceptions regarding color has been rather figurative or from an artist's viewpoint This has been due to the lack of standards, and chiefly for the reason that color has not been expressed in terms of its three attributes relative luminosity (brilliance), dominant wavelength (hue) and purity (saturation)

TINTOMETRIC METHODS

Various tintometric or colorimetric methods of estimating color have been described in the literature (2, 3, 9, 13) Various color plaques are employed in such tests and are in character analogous to the Tallquist scale for the estimation of hemoglobin Such tintometric and colorimetric methods of estimating and recording color are doubtless of value to clinical medicine, especially when subjected to some definite system of color analysis, such as the Munsell disk or the Bradley top However useful such methods may be, they are open to the objection that they do not in any wise analyze the spectral light reflected by the skin and therefore cannot record in terms of the three fundamental attributes of color Furthermore, pigments and spectral colors are vastly different The spectral colors of white light, such as sunlight, are pure colors, can be measured as to intensity, purity, and so on, and are, therefore, readily standardized For instance, spectral yellow of a given wavelength (for example, 590 millimicrons or 5900 Angstrom units) is always the same yellow and can be reproduced exactly

ATTRIBUTES OF COLOR

White light can be made spectrally from certain quantities of red, green and blue of limited wavelength values or, again, from certain quantities of orange, green and blue of limited wavelength values according to the tables of Maxwell (7) and others (10) Unless a color is described according to its attributes, nothing definite is stated regarding the color The three attributes of color are relative luminosity, dominant wavelength and purity Relative luminosity or brilliance is necessary if color exists at all It is often spoken of as brightness, tint, value, or visual brightness In this particular attribute,

colors may be classified as equivalent to some member of a series of grays of which black and white are the extremes   Relative luminosity simply tells how much of a standard source of light (such as sunlight) the given color is capable of reflecting   By dominant wavelength or hue is meant that attribute of the color which permits it to be classed as reddish, yellowish, greenish or bluish   The hue or tone depends on the wavelength only   Percentage of purity or saturation determines the degree of hue, stating how vivid or distinct it is Thus the percentage of purity defines how red or how yellow, and so forth, a color is   For a full discussion of these attributes of color and allied topics, reference should be made to the report of the Committee on Colorimetry of the Optical Society of America (14)

Since the pigmentation of the skin is largely due to melanin (at least in brunets, negroes and those suffering from Addison's disease), the degree of pigmentation, which affects the brilliance and purity only, may be thought of roughly as a smoke screen (in some persons very slight and in others very dense) laid down between solar radiation, on the one side, and the blood in the peripheral capillaries on the other side of the dividing medium, the epidermis   In other words, the presence of a pigment, such as melanin, will not affect the dominant wavelength but will influence the relative luminosity and saturation On the other hand, the presence of abnormally large quantities of blood at the periphery of the body may influence the dominant wavelength and perchance also the other attributes of the color of the skin   The effects of pigmentation and superficial blood are the subjects of the second and third papers of this series dealing with the color of the skin

It is readily seen, therefore, in a consideration of the color of the skin (which is a semi-opaque body) that systems dependent on color matching with the human eye fail to analyze sufficiently the light reflected from the skin   It is physically impossible for the human eye to estimate color quantitatively and to analyze it according to its attributes   Rowntree and Brown (9) developed a universal skin tintometer, adaptable to clinical medicine, which consists of nine separate color scales   Matchings were made and grades given to various selected areas of skin under conditions least likely to introduce error   These readings were then subjected to the Munsell (8) system of color measurement

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briefly the essential points of construction and operation   A spherical lamp house lined with zinc-oxide paint and holding two 400 watt stereopticon bulbs serves as a source of white light which remains
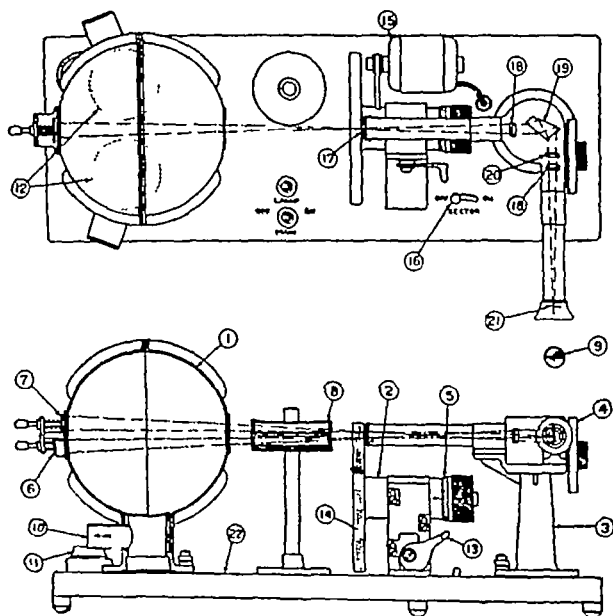
FIG 2 CROSS-SECTIONAL DIAGRAMS SHOWING THE WORKING PARTS OF THE SPECTROPHOTOMETER

1, spherical light house, *2*, photometer, *3*, spectrometer, *4*, wavelength scale, *5*, photometric scale, *6*, holder for standard sample, *7*, holder for reflection samples, *9*, field of view through the eye slit, *14*, sector disks, *17*, entrance slit, *19*, dispersion prism, and *20*, biprism

constant during all the readings   Smooth magnesium blocks at the rear of the sphere serve to reflect the light through various transparent mediums (liquids) which may be placed in the path in the support (fig 2)   In such experiments as these, in which an opaque object

such as the skin is to be studied, the holder for liquids is not used
One of the magnesium blocks is replaced by the area of skin to be
investigated   We have recently devised a substitute for the regular
attachment to the spectrophotometer which is water-cooled and
which enables any portion of the body to be examined   This device
will be described in subsequent paragraphs   The two beams of light,
reflected from the magesium block and the skin, respectively, enter
the spectrophotometer to be analyzed   The spectrometer is essen-
tially the ordinary constant deviation type of instrument except for
the addition of a biprism and an observing slit at the exit   The spec-
trometer is set at any desired wavelength by rotating the wheel, 4,
which serves to turn the prism of the spectrometer   The amount of
light admitted to match brightness or saturation value is determined
by the variation in the sector openings in the rotating disks in the
photometer   This is controlled by a calibrated drumhead, 5   The
image at the observing exit slit consists of two semicircular colored
fields separated by a horizontal line   The hue of these areas will be
the same and will depend on wavelength only   Equality of brightness
can be obtained by turning the photometer drumhead until an exact
match is obtained   This reading (0 to 100 per cent) is then recorded as
the percentage of light reflected (or transmitted) at the particular
wavelength at which the spectrometer is set   Throughout this
particular series of observations the entrance slit was adjusted to give
constant reflection of light from the source of white light throughout
the whole spectrum, and the entrance and exit slits were thereafter
maintained at fixed values

Readings are taken of the specimen under consideration by turning
the calibrated wheel, 4, over a space of 10 to 20 millimicrons   The
percentage of light reflected at each wavelength is read directly
from the matching of brilliance obtained by turning the drumhead, 5,
at the same time   A spectrophotometric curve can then be plotted,
using wavelengths of light as the abscissas and the percentages of
reflection as the ordinates   This gives graphic data which are readily
transferable into other values, as will be shown   From such a spec-
trophotometric curve an experienced observer can determine certain
characteristics of the object under consideration by studying the form
of the reflection curve in particular regions of the spectrum   Such
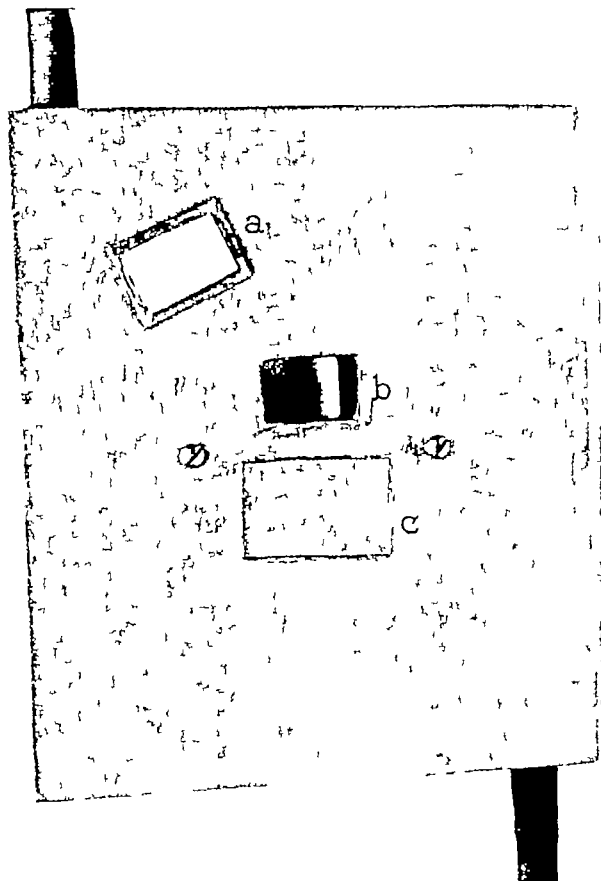
FIG 3 WATER CELL ATTACHMENT TO REPLACE USUAL HOLDERS FOR STANDARD
AND SUBSTANCE TO BE EXAMINED BY METHODS OF REFLECTION

a brass plaque coated with magnesium carbonate *b*, aperture in the water
cooled holder which is attached to the instrument, and *c*, standard in position

spectrophotometric readings, since they measure the spectral distribution of the color of the object examined and so actually measure the light stimuli, are independent of the condition of color vision of the observer's eye Therefore, all observers should get the same results Also, when a colored object is viewed by the same observer with the naked eye under lights of different quality, the sensation produced under each light will be different although the color itself has not changed physically Measurements made with a spectrophotometer
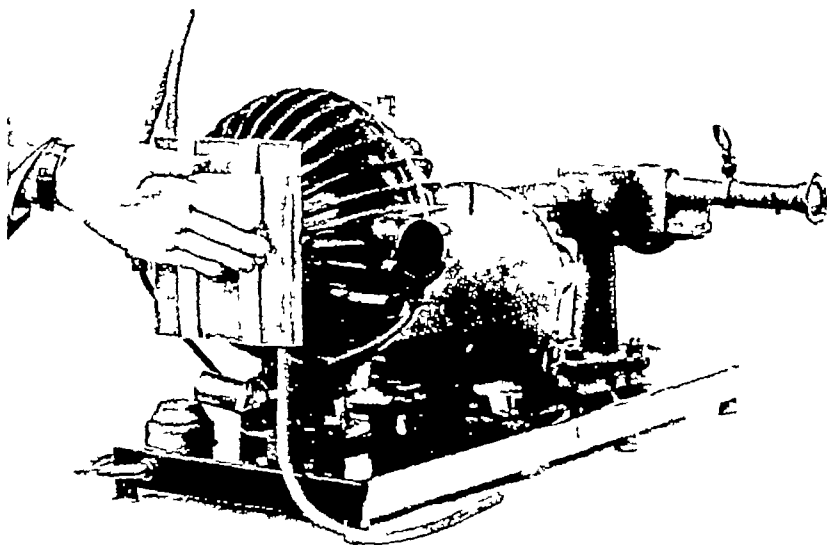
Fig 4 Keuffel and Esser Color Analyzer with Water Cell Modification Attached, the Relation of the Instrument to Sample to Be Studied Is Shown

are independent of the quality of the source of light as contrasted to color observed by the method of colorimetry or direct matching

AUTHORS' WATER-COOLED DEVICE TO PERMIT SPECTROPHOTOMETRIC EXAMINATIONS OF LIVING MATERIALS

The Keuffel and Esser color analyzer was originally adapted to record reflection values from such opaque flat objects as paper, cloth, leather, soap flour paints, and the like, and to serve as a basis of color

specification and color control   To adapt the apparatus so that areas of human skin could be measured, it was found necessary to modify the specimen holder   Such easily available portions of the body as the back of the hand or the fingers could be read with the original apparatus, although it was necessary to allow time for the cooling of the lamp-housing in spite of the fact that a current of air was allowed to play between the lamps and the specimen being examined   In devising a practical specimen holder it was borne in mind that although the distance from the source of light could be increased to eliminate to some extent the effects of heat, the illumination suffered in direct proportion   A solution to the problem was found in a thin metal water cell (figs 3 and 4) so constructed as to fit in the place of the ordinary holders and be directly attached to the spherical lamp-housing   The circulation of the water serves to maintain the cell at a constant cool temperature   Small rectangular perforations are cut into the face of the cell, one above the other   The distal surfaces are covered, in the lower case by a white reflecting surface to take the place of the magnesium block in the original standard. The sample to be tested is brought flush against the upper opening, separated from the metal only by a thin layer of felt which is conveniently changed for purposes of cleanliness and which acts to prevent a change of temperature in the skin   A small, removable plaque, faced on the one side with white magnesium carbonate paint, was constructed and placed in the aperture ordinarily used for reflection purposes in case the instrument was to be used for transmission spectral studies   It is necessary to take the same precaution with the white facings on these metal plaques as with the standard magnesium blocks, for they must be kept smooth and clean throughout.   They can be checked at the beginning of any particular experiment to determine whether the reading of brilliance on the sector scale is approximately 100 per cent

CONVERSION OF SPECTROPHOTOMETRIC DATA INTO TERMS OF COLOR EXCITATION VALUES

In order to convert the curves of spectral reflection (or transmission) into a form which can be interpreted directly in terms of color excitation values, it is necessary to make certain computations   Probably

the most fundamental of all psychophysical data relating to color are the three color excitation curves which represent graphically the laws of a three-color mixture   The principal data on these relationships we owe to Maxwell (7), Abney (1), and Konig and Dieterici (6) The results of the latest investigations on this matter, reduced to an equal energy spectrum and referred to average noon sunlight, are plotted in figure 5

Spectrophotometric data are given generally in the form of curves of spectral transmission or reflection   Such curves require combina-
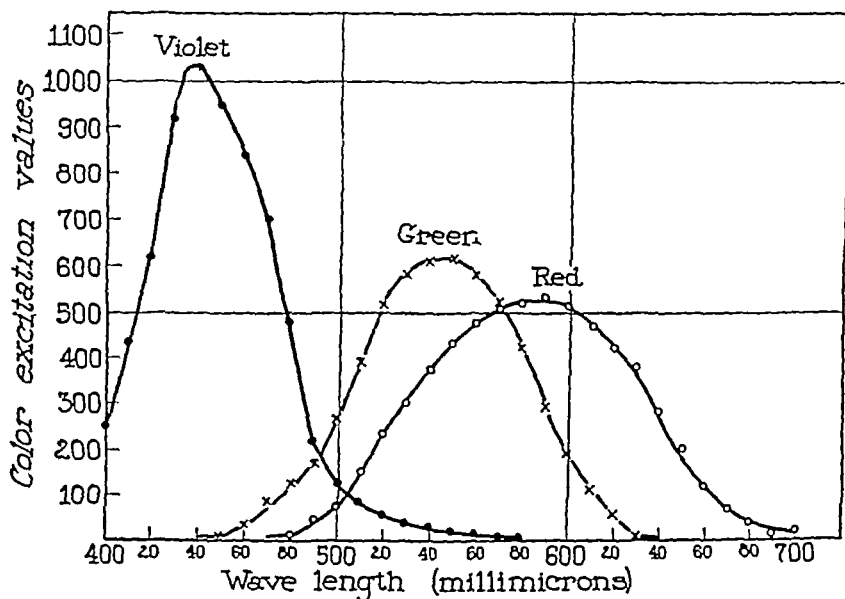
FIG 5 ELEMENTARY COLOR EXCITATIONS FOR DIFFERENT WAVELENGTHS

tion with a certain energy distribution representative of the particular source with which the object is illuminated in order that they shall become determinative of a definite color   The process of reducing any given set of spectrophotometric data to excitation color values is therefore as follows (1) multiply each of the ordinates of the transmission or reflection curve by the corresponding ordinate of the energy distribution curve of the source, (2) multiply each of the ordinates of the resulting curve by the corresponding ordinate of each of the color excitation functions as shown in figure 5, this being a separate opera-

tion for each of the three excitations and yielding three separate curves which represent the respective excitation values for each wavelength of the given stimulus, (3) determine separately the areas of the three curves thus found, and (4) reduce the three areal values thus obtained to percentage form, so that their determined ratio re-
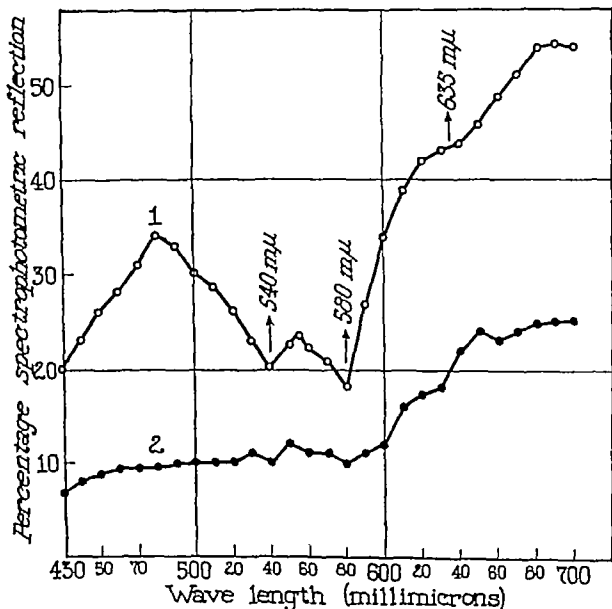
FIG 6 SPECTROPHOTOMETRIC CURVES OBTAINED BY REFLECTION FROM THE FINGERS

Curve 1, normal blond, curve 2, normal mulatto

mains unchanged but their sum becomes equal to 100   The color excitation values can then be expressed by means of two numbers, representing the red and violet excitation percentages, that for the green being obtainable by subtracting the sum of these two values from

100   Complete details on these points are given in the report of the Committee on Colorimetry of the Optical Society of America (14)
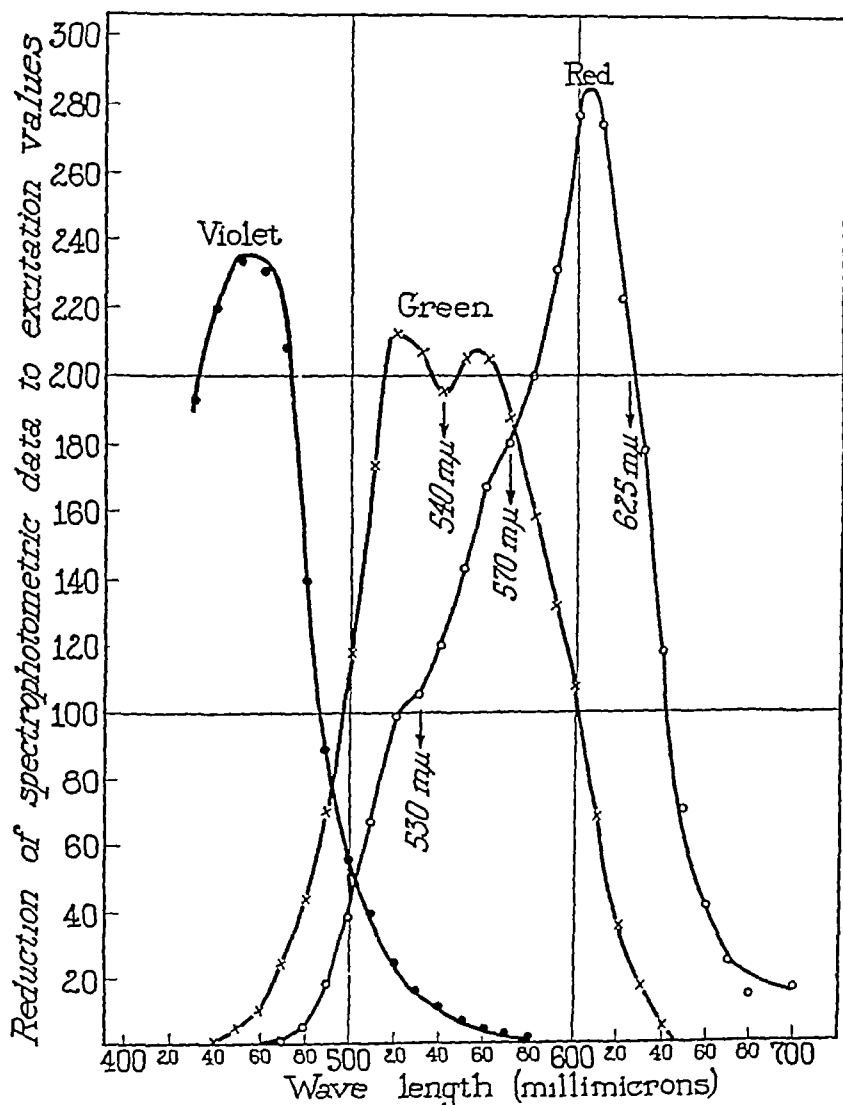
FIG 7   ANALYSIS OF THE SPECTROPHOTOMETRIC DATA (SHOWN GRAPHICALLY IN FIGURE 6) INTO THE THREE PRIMARY EXCITATION CURVES

Figure 7 contains the red, green and violet excitation curves for the spectrophotometric data given in curve 1 of figure 6, which was

obtained in the case of a young normal blond   This curve of spectrophotometric reflection, as well as that which gives the subsequent analysis of this reflection curve into fundamental red, green and violet
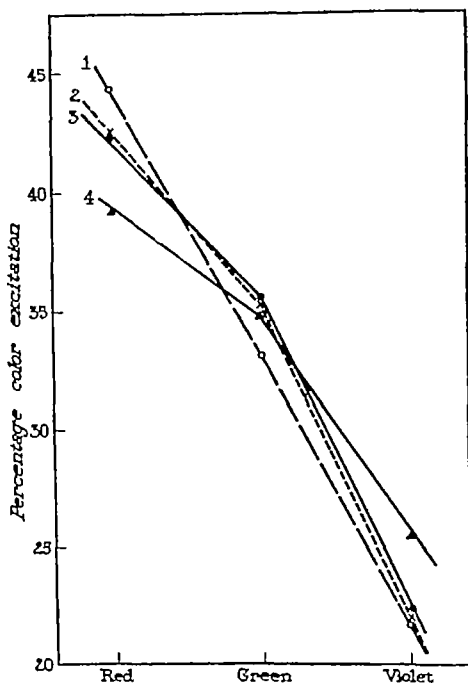
FIG 8 PERCENTAGE COLOR EXCITATION VALUES

Curve 1, normal brunet, curve 2, a case of Addison's disease, curve 3, a normal negro, and curve 4, a case of scleroderma   All data were obtained from fingers held slightly below heart level

color values, shows the presence of spectral bands at 625, 580 and 540 millimicrons respectively   The bands at 580 and 540 millimicrons are due to the absorption of light by the blood (oxyhemoglobin bands),

and the band at 625 millimicrons may be attributed to the presence of hematin in either the skin or the blood, or possibly both   The conversion of the spectrophotometric curves into color excitation
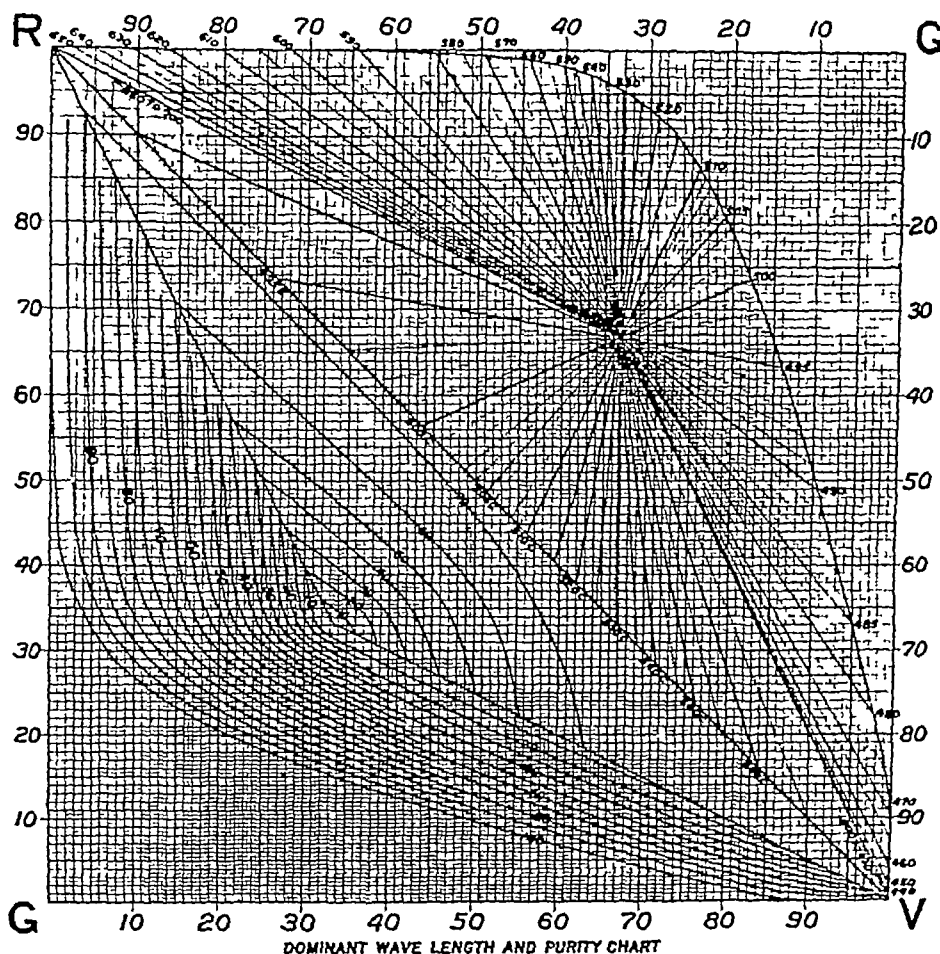
FIG 9 DOMINANT WAVELENGTH AND PURITY CHARTS FROM WHICH THE HUE AND THE DEGREE OF SATURATION MAY BE OBTAINED BY THE USE OF DATA FROM THE PERCENTAGE EXCITATION CALCULATIONS

values, as outlined in the preceding paragraph, is much simplified and rendered more rapid by the use of a special color slide rule prepared by Keuffel and Esser (Hoboken, New Jersey) for this purpose

The summation of the values of the color excitations for the three

fundamental colors, red, yellow and violet, respectively, proceeding from 700 to 430 millimicrons (for example, the separate excitation color curves shown in figure 7) gives the total red, green and violet excitation values respectively   From the sum of the three separate summation values it is easily possible to obtain the percentage of red, green and violet color excitations, respectively   Figure 8 gives a group of such percentage color excitation values in the case of a normal brunet (curve 1), a case of Addison's disease (curve 2), a normal negro (curve 3) and a case of scleroderma (curve 4)   In the subsequent papers which deal with the rôle of pigmentation and of superficial blood a number of such analyses will be presented and discussed

In addition to the data regarding the total color excitation values and the relative percentages of red, green and violet, respectively, in the white light reflected by the skin, the dominant wavelength or hue and the percentage purity or saturation can be determined by the use of charts provided for this purpose   A reproduction of such a chart which may be obtained in large dimensions from Keuffel and Esser, is shown in figure 9   Using this color triangle and taking the percentages of red and violet determined from an analysis of any spectrophotometric curve into its fundamental red, green and violet components, we are able to find the dominant wavelengths (or hues) as well as the degrees of purity and relative luminosities

SUMMARY

In this paper we have presented and briefly commented on (1) tintometric methods for estimating the color of the skin, (2) the fundamental attributes of color, (3) spectrophotometric apparatus and methods of procedure for obtaining curves of the reflection, by the skin, of light of various wavelengths, (4) the authors' water-cooled device to enable spectrophotometric determinations to be made on living materials, (5) spectrophotometric analyses of the color of the skin, and (6) conversion of spectrophotometric data into terms of color excitation values, percentages of fundamental red, green and violet reflected by the skin, dominant wavelength, purity and relative luminosity

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THE COLOR OF THE SKIN AS ANALYZED BY SPECTRO-PHOTOMETRIC METHODS

II THE RÔLE OF PIGMENTATION[1]

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The color of the skin of human beings depends chiefly on the presence of pigment and superficial blood Spectrophotometric data on the reflection of visible light from the surface of the skin were obtained in a number of subjects possessing various degrees of pigmentation Analysis of the spectrophotometric curves obtained for normal and pathologic subjects into terms of red, green and violet excitation color values, and subsequent conversion of these values into expressions of dominant wavelength, purity and relative luminosity, indicate certain fairly constant relations of content of pigment to the color of the skin

Certain secondary factors contributing to the amount of light reflected from the skin, such as surface dirt, oil and moisture, were eliminated by making reasonably sure that the area under investigation was clean Preliminary readings apparently showed that the presence of a small amount of hair or the obliteration of the normal rugae of the surface of the skin did not affect the percentage of light reflected to any considerable extent. Only under pathologic conditions, involving especially the presence of an abundance of scales, were the reflection values materially altered It may be said in general that the local contour may be disregarded as a factor in affecting the color of the skin

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[1] The material in this paper was submitted by Dr Brunsting to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Dermatology and Syphilology 1929 Work done in the Division of Physics and Biophysical Research

Schultze (4) attempted an analysis of the utilization of light by the skin as manifested by its spectral reflection and transmission He used a photographic-photometric method of recording the light reflected by the skin, further analyzed the results by means of a Marten's polarization photometer, and plotted the curves in percentages of reflected light The results of Schultze give information regarding the total reflection of visible light by the skin in various areas of the body under controlled conditions Although the results do not embody a monochromatic analysis of the reflected light, they show that pigmentation tends to diminish the amount of light reflected by the skin

Dorno (2), using similar methods observed variations in the reflection of light by the skin as affected by race, age and sex He likewise noted general diminution of the light reflected under conditions of increased pigmentation

Sonne (8) estimated that the skin of a normal person was capable of reflecting approximately 35 per cent of the visible light falling on it

Sheard (5), and Sheard and Brown (6) were among the first to apply spectrophotometric methods to clinical medicine in a series of observations on the color of the skin in various normal and pathologic conditions, and were probably the first to analyze the resultant spectrophotometric reflection curves into percentages of fundamental red, green and violet excitation color values, dominant wavelength and saturation The series of investigations reported here is essentially a continuation of their work

The direct-reading spectrophotometer, known as the Keuffel (3) and Esser color analyzer, with the author's water-cell modification (1), was used throughout these investigations The cleaned surface of skin was brought flush against one aperture of the water-cooled cell, with constant conditions of temperature and pressure Readings were made directly in terms of the percentage of light reflected from the surface

In order to evaluate the rôle of pigment as a factor in contributing color to the skin, comparative records were obtained on subjects presenting varying gradations of pigmentation under normal and abnormal conditions The following distinctions were observed (1) variations over several areas of the body of the same subject, (2)

variations among different subjects classified according to type, as blond or brunet, (3) variations in subjects of different races, including the so-called white, black, yellow and red races, (4) a comparison of normal values with the data obtained for subjects manifesting abnormal degrees of pigmentation, such as in Addison's disease, arsenism, argyria and jaundice
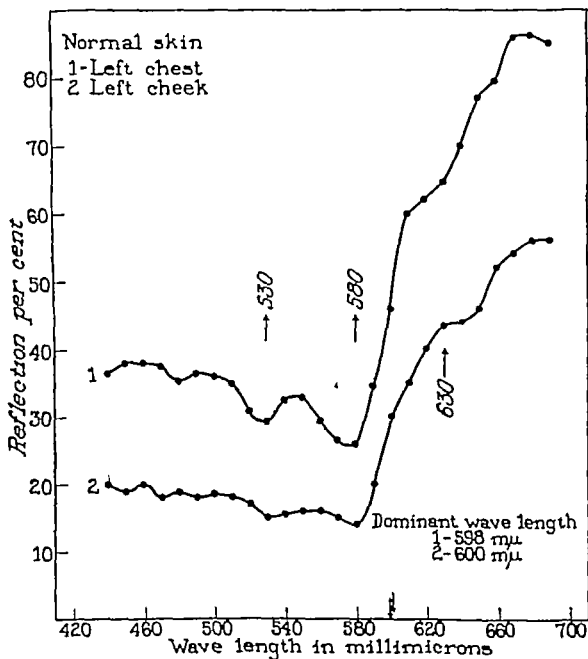
Fig 1 Spectrophotometric Reflection Curves of the Skin of a Normal Subject *1*, Chest, *2*, Cheek

VARIATION IN PIGMENTATION IN THE SAME SUBJECT

It is self-evident that the normal skin presents an amount of pigment roughly proportional to the amount of habitual exposure to the

sun and the elements   There is a wide variation in pigmentary
response in the same subject, as well as in different subjects, to expo-
sure to sunlight

Figure 1 represents two curves from the same subject showing a
record of the light reflected from the skin of the cheek as compared
with the chest   In general form the curves are similar, that is, they
maintain a similar linear relationship   However, there is a constant
difference in the percentage of light reflected throughout the visible
spectrum, more being reflected from the chest (which is ordinarily
protected from sunlight, as in this instance) than from the cheek,
which is more or less exposed   This difference in the amount of re-
flection represents a distinction in relative luminosity or brilliance of
the areas under consideration   The curves show decreased percent-

TABLE 1

Analysis of color values obtained from spectrophotometric curves of figure 1

Curve	Area	Total color excitation	Red	Green	Violet	Dominant wave-length	Purity	Relative luminosity
		units	*per cent*	*per cent*	*per cent*	*milli microns*	*per cent*	*per cent*
1	Chest	6,708	41 5	32 7	25 8	598	26	36 7
2	Cheek	3,703	44 1	31 7	24 2	600	33	20 5

ages of reflection at the regions corresponding, approximately, to 540,
580 and 630 millimicrons, respectively   That these are related to the
presence of superficial blood seems likely, as stated by Sheard and
Brown (6), absorption at 540 and 580 millimicrons is due to oxyhemo-
globin and at 630 millimicrons is probably due to hematin   Zones
of decreased reflection, as shown in the curves, are more pronounced
in the reflection curve obtained from the surface of the chest than from
the cheek, inasmuch as the amount of pigment interposed is corre-
spondingly less   The influence of pigment on these absorption
bands is to minimize them   This will be brought out more fully later
in the paper

Table 1 represents a summary of data obtained by an analysis of
the spectrophotometric curves in figure 1, following the methods
outlined by the Committee on Colorimetry of the Optical Society of

America (10), these methods consist of the translation of reflection values into terms of color, namely hue or dominant wavelength, purity, and brilliance or relative luminosity In general, the quantity and quality of color are expressed in terms of stimulus or color excitation units, which include the summated values of the red, green and violet These values determine the index of saturation or purity of hue as well as the dominant wavelength The exact procedure used in the analyses is discussed elsewhere (1, 7)

Pigmented skin reflects less light than nonpigmented skin, and stimulates fewer color excitation units, giving a diminished percentage of total brilliance The skin of the cheek, in this instance, has a relative luminosity value of only 20 5 per cent, as compared to 36 7 per cent for the skin of the chest. The relative percentages of red, green and violet are an index to the hue and the degree of hue, saturation or purity In this instance there appear to be higher percentages of red and lower percentages of green and violet color excitation values obtained from the cheek than from the chest and correspondingly different percentages of purity Nevertheless, the red, green and violet values in each instance maintain a linear relationship in spite of the difference in color excitation units produced by the two areas under consideration This holds true regardless of changes in the quantity of pigment present

VARIATIONS AMONG DIFFERENT SUBJECTS CLASSIFIED ACCORDING TO
TYPE AS BLOND OR BRUNET

A series of blonds and brunets, selected at random, was investigated with reference to variations of pigment content of the skin of widely separated areas of the body Spectrophotometric reflection curves were obtained from the cheek, chest, inner side of the arm and the dorsum of the middle finger, the hand in the last instance being held about 18 cm above heart level to reduce the quantity of the superficial venous blood These curves were analyzed in each instance into the attributes of color, and the resultant data are given in table 2

Quantitative estimates of the intensity of pigmentation can be made readily from these data The individual values for the amounts of red, green and violet are maintained at a fairly constant level. This results in little, if any, change in the hue or dominant wavelength,

which remains approximately at 590 millimicrons   This corresponds to pure spectral yellow   Likewise the purity or saturation is a func-

TABLE 2

Analyses from spectrophotometric curves of normal blonds and brunets to demonstrate variation of pigment over body areas more or less exposed to light

Case	Type	Age	Sex	Area	Total excitation	Red	Green	Violet	Hue	Purity	Relative luminosity
					units	*per cent*	*per cent*	*per cent*	*milli-microns*	*per cent*	*per cent*
1	Blond	28	F	Chest	7,295	41 0	33 0	26 0	595	27	39 0
				Hand above heart level	6,812	40 1	32 3	27 6	600	21	36 3
2	Blond	22	F	Chest	8,202	39 2	34 3	26 5	587	26	44 3
				Hand above heart level	6,775	39 3	34 4	26 3	587	26	36 9
3	Blond	18	F	Chest	8,422	39 5	34 2	26 3	590	27	46 1
				Hand above heart level	7,848	40 0	33 8	26 2	590	27	42 7
4	Blond	32	M	Chest	8,579	39 0	34 1	26 9	590	24	44 2
				Cheek	5,973	40 3	32 8	26 9	598	23	33 0
5	Brunet	35	M	Inner side of arm	6,924	43 9	34 6	21 5	590	43	41 7
				Cheek	4,372	41 2	34 0	24 8	590	31	24 3
6	Brunet	36	M	Inner side of arm	6,601	41 3	35 2	23 5	587	37	37 3
				Cheek	3,710	43 9	33 3	22 8	597	37	20 9
7	Brunet*	32	F	Chest	5,024	42 0	32 6	25 2	596	30	28 1
				Hand above heart level	5,706	41 3	34 0	24 7	592	28	32 4
				Cheek	4,904	41 2	32 8	26 0	596	27	26 8

* Much generalized tanning

tion of the amount of red, green and violet color excited   The blond types manifested a more constant percentage of purity, ranging from 21 to 37 per cent, than did the brunet types, whose percentage varied

from 27 to 37 per cent, with one value as high as 43 per cent   In general, as the percentage of red summation increased, the percentage of violet decreased, with very little variation in the percentage of green color   That is to say, the linear relationships were preserved in these types of light and dark complexions

The brilliance, however, underwent marked variation, and apparently in direct proportion to the absence of pigment present in the specimen of skin under observation   The amount of pigment de-

TABLE 3

Analyses from spectrophotometric curves of a Negro Chinese and Japanese

Type	Age	Sex	Area	Total excitation	Red	Green	Violet	Hue	Purity	Relative luminosity
				units	*per cent*	*per cent*	*per cent*	*milli microns*	*per cent*	*per cent*
Negro	42	M	Chest	3,316	42 4	33 8	23 8	590	35	18 6
			Hand above heart level	1 272	44 3	36 3	19 4	585	50	7 5
Chinese	40	M	Chest	6,329	39 8	35 9	24 5	585	33	33 7
			Malar region	5,753	42 9	34 0	23 1	590	33	32 0
			Hand above heart level	4,883	39 0	34 7	26 3	585	28	27 2
Japanese	30	M	Inner side of arm	5,240	39 5	33 0	27 5	595	22	28 0
			Hand above heart level	4 409	40 4	34 8	24 8	587	31	24 3

termines the total amount of the summated excitation values for red, green and violet   For instance, in the case of a brunet, skin from an area such as the chest or the inner side of the arm (which had received very little exposure to sunlight) would reflect in some cases more light than skin (blond) which had become heavily pigmented through constant exposure. Although pigment affects the brilliance of color, in and of itself, it does not alter the values of the dominant wavelength and purity   Blonds and brunets are undistinguishable except for relative variation in brilliance or luminosity

Variations in subjects of different races

The color of the skin has always held an important place among typical criteria of race   Records have been obtained on a number of subjects (fig 2), of which the values for a full-blooded negro, a Chinese and a Japanese are tabulated for purposes of comparison (table 3)

The skin of the negro reflects considerably less light than the skin of the Japanese or the Chinese   A percentage of relative luminosity
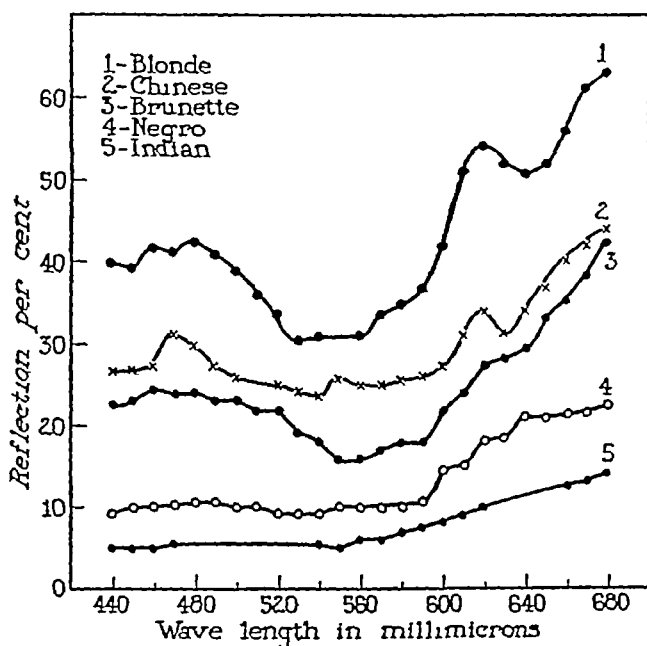
FIG 2   A SERIES OF SPECTROPHOTOMETRIC CURVES BY THE REFLECTION METHOD OBTAINED FROM THE SKIN OF SUBJECTS OF VARIOUS RACES

or brilliance of 7 5 was obtained in the area of skin from the dorsum of the hand of the negro   The surface of the chest, however, which was ordinarily covered, reflected 18 6 per cent of the light falling on it Moreover, the percentage values for red, green and violet appear to be in a different relation to each other   Nevertheless they have maintained the same linear relationship, as demonstrated in the skin of the white subject.   The purity of the color in the area of the hand reaches

50 per cent, but the hue or dominant wavelength remains constantly near the region of 590 millimicrons
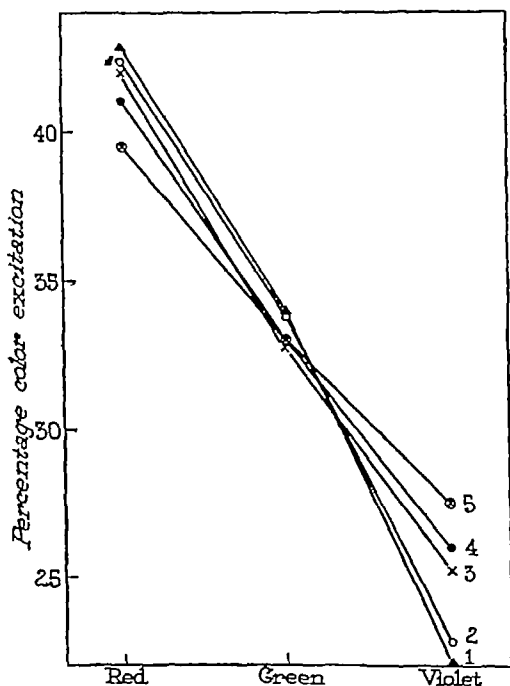
FIG 3 PERCENTAGE COLOR EXCITATION VALUES OBTAINED FROM SPECTRO
PHOTOMETRIC DATA IN PERSONS EXHIBITING VARYING DEGREES OF
PIGMENTATION 1, CHINESE, 2, NEGRO, 3, BLOND,
4, BRUNET AND 5, JAPANESE

Data obtained by the reflection method, after being analyzed into fundamental red, green and violet components for the blond, brunet, negro, Chinese, and Japanese have been plotted and are shown graphically in figure 3   On the basis of specification of color alone,

a definite distinction cannot be made as to whether a subject is a member of one race or another

Todd and Van Gorder (9) in anthropologic studies on the skin of the American negro, utilized the system of spinning tops (Bradley) to estimate the composite coloring of the skin with particular regard to the content of the black pigment This work was part of a larger research on the correlation of various physical features and was presented as an effort to promote greater accuracy in the quantitative estimation of the color of the skin The method does not provide for the analysis of color as defined according to its attributes of hue, purity and relative luminosity, but tabulates the records obtained in the registration of brightness, or lack of it, in the skin of the negro Our work confirms the impression of these authors that the color of the skin is a faulty racial test when used without regard to other physical features of the body

We have had the opportunity of making spectrophotometric examinations of the color of the skin of a group of native American Indians of three distinct tribes As far as could be determined by physical inspection, or ascertained by direct questioning, these Indians were free from an admixture of white or negro blood Table 4 presents an analysis of data obtained from reflection curves of the skin of various parts of the body in subjects of both sexes and of different ages

The American Indian has long been known as a member of the "red" race This name seems to have been given the race because of the custom of smearing their faces with ochre and not because of any redness in the skin itself Clinical inspection of the skin of the American Indian suggests a color resembling bronze As far as is known to us, an attempt has not been made previously to analyze the color of the Indian skin according to accurate standards of color

The uniformity of results obtained in the data representing fundamental color values, as shown in table 4, is of particular interest The various areas of skin observed show a degree of brilliance or relative luminosity inversely proportionate to the density of the screen of pigment present This relationship was demonstrated similarly in an analysis of the other racial types, namely, the white, black and yellow The hands and face are, of course, much darker than such

TABLE 4

Analyses from spectrophotometric data in American Indians

Tribe	Age	Sex	Area	Total excitation	Red	Green	Violet	Hue	Purity	Relative luminosity
				units	per cent	per cent	per cent	milli microns	per cent	per cent
Winnebago	23	M.	Chest	4,678	44 6	33 3	22 1	590–595	41 0	26 8
			Malar region	3,508	41 8	35 9	24 3	590–595	34 0	19 6
			Hand above heart level	2,421	42 1	33 7	24 2	590–595	34 0	13 5
	21	M	Chest	5,210	42 7	35 2	22 1	585	41 0	29 9
			Malar region	3,993	38 9	31 1	30 0	620	11 0	20 8
			Hand above heart level	2,050	40 4	33 7	25 9	595	31 0	11 1
Kiowa	40	Step-father	Shoulder (protected)	5,394	41 2	34 2	26 6	590	33 0	29 9
			Malar region	3,268	44 1	31 1	24 8	605	30 0	17 7
			Hand at heart level	1,067	46 5	35 7	17 8	587	55 0	6 5
	45	Mother	Breast	4,622	42 8	33 1	24 1	596	32 0	25 8
			Malar region	3,770	43 5	32 3	24 2	600	33 0	20 9
			Hand at heart level	1,905	40 5	34 2	25 3	590	30 0	10 4
	18	Son	Malar region	4,035	41 2	33 8	25 0	590	30 0	22 2
			Hand at heart level	1,851	44 6	33 8	21 6	590	43 0	10 4
	24	Daughter	Shoulder (protected)	3 015	44 8	32 9	22 3	595	39 0	16 7
			Malar region	4 217	41 5	32 9	25 6	595	28 0	23 1
			Hand at heart level	2 377	35 8	33 5	30 7	595	9 0	11 6
Osage	32	Father	Malar region	3 904	38 5	32 4	29 1	605	15 0	20 4
			Hand above heart level	2 480	37 3	33 3	29 4	600	14 0	13 0
	27	Mother	Malar region	3 843	39 8	31 1	29 1	620	15 0	19 9
			Hand above heart level	3,201	40 2	31 3	28 5	619	17 0	17 6

TABLE 4—*Concluded*

Tribe	Age	Sex	Area	Total excitation	Red	Green	Violet	Hue	Purity	Relative luminosity
				units	*per cen*	*per cent*	*per cent*	*milli-microns*	*per cent*	*per cent*
Osage	10	Son	Malar region	5,838	37 0	32 7	30 3	605	11 0	29 3
			Hand at heart level	3,045	39 2	33 9	26 9	590	24 0	16 4
	7	Son	Malar region	6,117	38 0	33 5	28 5	595	18 0	32 5
			Hand at heart level	2,737	39 4	34 0	26 6	590	26 0	14 9

protected areas as the trunk   This would be expected from the difference in habitual exposure to sunlight

The hue or dominant wavelength throughout the entire series of Indians averaged 593 millimicrons, if the malar areas are excluded The malar areas in most of the cases examined appeared to contain a little more red, in one case attaining 620 millimicrons   The average was 603 millimicrons   This departure may be because of some anatomic peculiarity of the skin in the malar area, especially as regards its texture, which may allow an undue influence to be exerted on the color of the skin by the superficial capillaries and telangiectasia so commonly found in this region

The subjects from the Winnebago and Kiowa tribes revealed a level of hue which was similar to that recorded previously for other subjects regardless of race   The values of purity are relatively high except in two instances, namely, in the malar area of the second Winnebago Indian (11 per cent) and in the hand of the fourth Kiowa Indian (9 per cent)   In these cases it is possible that the presence of superficial blood may have affected the readings obtained   The relative luminosity or brilliance is uniformly low, as would be expected, since the skin of the Indian, like that of the negro, presents a dark surface and hence reflects little light   In no case did the brilliance exceed 30 per cent in the lightest areas, and in one case (in the hand of the first Kiowa Indian) it dropped to 6 5 per cent, the lowest level of any of our records

The Osage Indians were a family group and presented a few variations distinguishing them from the other types of Indians, although perhaps not characteristically so   The dominant wavelength lies in the orange region of the spectrum, in one instance at 620 millimicrons   The relative proportions of red, green and violet show a change in the linear relationship by a lowering of the value for red and some-
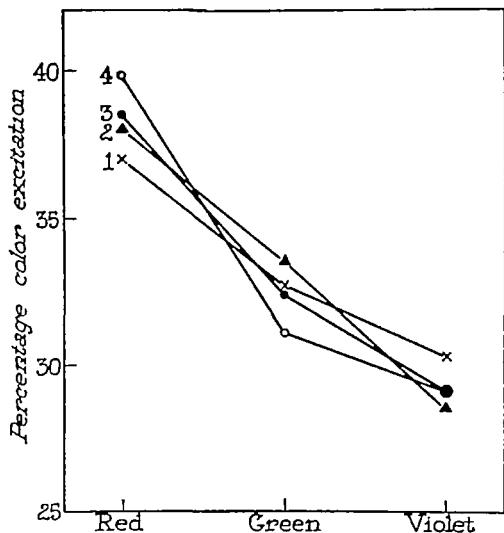
FIG 4 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC
DATA IN THE OSAGE INDIAN FAMILY GROUP *1*, SON, *2*,
SON, *3*, FATHER, AND *4*, MOTHER

times an exaggerated increase in the value for violet   A break in the linear arrangement of these percentage values is reflected, in turn, in the values of the dominant wavelength and purity

Figure 4 is a graphic representation of the percentage values resulting from an analysis of the reflection curves obtained in the four members of the Osage Indian family   Curves 1, 3 and 4 show a slight

tendency to deviation from a straight line as drawn through the specified value of the green component This is especially so in curve 4, which is the record of color values from the malar area of the

TABLE 5

Analyses of spectrophotometric data in subjects presenting pigmentary disturbances

Type	Age	Sex	Area	Total excitation	Red	Green	Violet	Hue	Purity	Relative luminosity
				units	per cent	per cent	per cent	milli microns	per cent	per cent
Argyria (confirmed clinically and microscopically)	40	M	Breast	8,094	37 2	34 2	28 6	586	18	43 0
			Malar region	4,398	38 0	34 2	27 8	587	21	23 5
			Hand at heart level	3,064	35 8	33 6	30 6	590	10	15 5
Addison's disease	36	F	Chest	5,735	36 7	33 4	29 9	590	12	30 0
			Malar region	3,467	41 9	34 0	24 1	590	34	20 0
			Hand at heart level	2,425	40 9	32 4	26 7	590	25	13 0
Arsenism	40	M	Back (pigmented)	5,354	41 6	34 9	23 5	587	36	30 3
			Malar region	4,274	42 4	33 2	24 4	595	32	22 6
			Hand at heart level	2,787	43 7	34 5	21 8	590	41	16 6
Arsenism	42	M	Back (pigmented)	5,863	42 9	36 0	21 0	585	45	34 6
			Forehead	5,084	43 3	34 0	22 7	590	40	28 8
			Hand above heart level	3,707	45 7	34 3	20 0	590	45	21 7
Jaundice	43	M	Malar region	5,156	41 8	33 3	24 9	595	30	28 4
			Hand above heart level	5,371	37 3	35 4	27 3	580	24	28 3

mother of the family Curve 2 is a straight line, being similar in this respect to all the previous readings obtained on subjects regardless of race or degree of pigmentation Subsequent readings in other conditions have indicated that the variations presented by the curves 1, 3 and 4 are indicative of the influence exerted by the superficial

blood on the color of the skin   This will be dealt with in greater detail in study III of this series   We may assume that the skin of this group of Osage Indians, being of unusually fine grained and thin texture, allowed greater penetration of the surface blood as a factor in influencing its color

VARIATIONS IN SUBJECTS MANIFESTING ABNORMAL TYPES AND DEGREES OF PIGMENTATION

Further data were obtained on types of pigmentary disturbances in order to permit of a comparison of these data (table 4) with similar analyses of subjects showing normal variation in pigmentation according to exposure to sunlight or to racial type alone   The results are given in table 5

The argyria was evident on clinical inspection and was of many years' standing   Silver nitrate had been administered for the treatment of ulcer over a period of several years   The diagnosis of argyria was confirmed by histochemical studies on a section of skin removed from the neck   Analysis of the curves obtained does not indicate any marked variations from the gradations of normal pigmentation previously investigated   The dominant wavelength remains at 586 to 590 millimicrons, the percentage of relative luminosity varied according to the amount of habitual exposure of the part, although a value of 43 per cent in the region of the breast indicates lack of pigment, either of melanin or silver   The purity or saturation tends to be fairly low and this may be related to the metallic luster characteristic of argyria

The values for Addisonian and arsenical pigmentation are not different from those obtained in subjects exhibiting normal variations in pigment.   The amount of brilliance recorded by the two cases of arsenism is of interest   The areas of skin on the back were particularly pigmented and were found to be the darkest areas on the body   However, in each case, values were obtained showing a reflection of more than 30 per cent of the total light falling on the surface   This bears out the statement made previously that the eye is a faulty instrument for analyzing the quantity and quality of color

Records were obtained from two subjects with jaundice and the curves analyzed as in the previous instance   The intensity of the

jaundice was quite pronounced, in each case quantitative estimation of the serum bilirubin by the van den Bergh technic showed 15 to 20 mgm for each 100 cc of blood The spectrophotometric data in these instances, when analyzed into the fundamental attributes of color, show that jaundice of itself does not alter the normal relations of the color of the skin The percentages of red, green and violet color and the hue, purity and relative luminosity are practically identical in the subjects with jaundice and in the Japanese and Chinese (table 3) It is reasonable to suppose, however, that degrees of jaundice will reflect themselves in the purity or saturation value of the hue predominating in the skin However, it is quite evident that the fundamental hue of the skin is not altered by subsequent deposits of bilirubin such as occur in jaundice, without regard to the intensity of the process

Staining of the tissues of the body by other pigments is occasionally seen after excessive ingestion of such substances as carrots, spinach and egg-yolk, which contain a relatively high percentage of lipo-chromes such as xanthophyll and carotin These pigments play an important part in the coloration of leaves and flowers and undoubtedly also contribute the yellow color to animal fat such as that which is present in the subcutaneous tissue Preliminary investigations on subjects giving evidence of coloration of the skin from such products indicate that the spectrophotometric data obtained will be exactly similar to the records of the subjects with jaundice

Pigment is one of the few substances which is enjoyed in common by plants and animals In plants there is a zonal distribution of color depending on the amount of exposure to the direct rays of the sun In seaweeds, for instance, there is gradation from a green color on the surface, through olive, orange and red as the dimly lighted ocean bottom is approached This affords an admirable adaptation to environment It may be true in animals, as in plants, that the pigment on the surface of the body acts in some degree as a regulator of metabolism The fundamental hue of the human skin remains constant in the spectral region of 590 millimicrons regardless of the amount of habitual exposure of the subject to sunlight Melanin is laid down as a screen, separating the external environment, on the one hand, from the blood and deeper tissues, on the other It may

act as a filter in regard to irradiation to which the surface of the body is exposed, possibly barring, by reflection, such fractions of energy as are injurious and unsuitable, and admitting by absorption such portions as are desirable, converting them into a form of energy convenient to immediate service or adaptable to storage for the future needs of the organism

CONCLUSIONS

1 Spectrophotometric analysis of the human skin demonstrates certain fairly constant features regarding its color

2 The fundamental hue or dominant wavelength of the skin lies in the spectral region 590 millimicrons (sodium yellow)

3 Deposition of melanin in the skin in response to more or less exposure to sunlight does not disturb the hue or purity of its color but decreases the relative luminosity The more melanin present, the lower the percentage of light reflected from the surface of the skin and the lower the brilliance

4 Pigment is not a racial characteristic. The same hue prevails in the white as in the so-called black, red and yellow races

5 Deposits of pigment in disorders of the skin do not alter the fundamental attributes of its color except as they lower the value for brilliance

6 Jaundice in the skin can be estimated quantitatively but it does not affect the normal values for hue, purity or relative luminosity

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THE COLOR OF THE SKIN AS ANALYZED BY SPECTROPHOTOMETRIC METHODS

III THE RÔLE OF SUPERFICIAL BLOOD[1]

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Variation in the superficial blood supply undoubtedly plays a more important rôle in the production of changes in the color of the skin than does an increase or decrease in the pigment   This accounts for the difference in subjects who are fairly well matched otherwise with regard to content of cutaneous pigment   In general, the pigment, in proportion to its amount, prevents the superficial blood from attaining visibility   Changes in color in the brunet, attributable to alterations in the circulation in the skin, are less noticeable than the same changes in the blond, and in the negro they are practically negligible   This holds true for the skin of the same thickness, it being quite obvious that the skin of the palms or soles or other hyperkeratotic areas will permit less light to penetrate than will the thin covering of the malar prominence or the dorsum of the hand

The color which the blood imparts to the skin is directly related to the amount or quality of the blood present in the peripheral vessels   The size of the arteries, capillaries, and venules varies with the degree of pressure behind them and with the volume of blood to be accommodated   In conditions of hypertension one would expect more force to be exerted peripherally than normally   In the dependent areas of the body, such as the legs and hands, the elements of gravity and stasis of sluggish flow are added   In cases in which the total blood volume

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and cells are increased, as in polycythemia vera, there is corresponding engorgement of all parts of the vascular tree, including the vessels of the skin, and an attempt is made to establish peripheral collaterals

Deviations from the normal oxygen unsaturation of the hemoglobin, as in cyanosis, cardiac or pulmonary decompensation, carbon monoxide poisoning, methemoglobinemia, and sulphemoglobinemia affect the color of the blood itself macroscopically, and so influence the color of the skin overlying it at any given time   Likewise, alterations in the serum content of various dyes, such as bilirubin in jaundice, and xanthophyll and carotin through intestinal absorption, reflect themselves to a certain extent in changes in the color of the skin

Physiologic variations in the area of exposure of surface blood for each unit of surface area of the skin are well known   Vasodilation and vasoconstriction occur following changes in the environment, such as heat and cold, and irritation locally by friction, or by the application of medicaments, or again following a disturbance of the emotions, as is seen in blushing or in blanching from fear

Quantitative estimations of the amount of exposed surface of the blood in polycythemia vera have been made by Brown and Sheard (1) by means of physical measurements obtained from projections of instantaneous photomicrographs of minute areas of skin   Computations in several cases of polycythemia vera tended to show that the surface capillaries occupied more than three times the allotment of surface area for the normal subject   Undoubtedly this accounts for a good deal of the change in color seen in such cases   Tintometric studies of color were also made but were not wholly satisfactory

The spectrophotometric measurements which we are presenting aim to show the relative components which enter into the color of the skin when it is subjected to variations in local blood content, either in quantity or quality   The water-cell modification of the Keuffel and Esser color analyzer was used in all the cases, special care being given to the area of skin under consideration to avoid extremes of temperature, which might produce local vasodilation or vasoconstriction, and also to avoid undue pressure locally, which might obliterate the superficial vessels to occasion blanching

Sheard and Brown (7) mentioned the presence of absorption bands

occurring constantly in the reflection curves corresponding to the special regions, 630, 580 and 540 millimicrons, respectively These were interpreted as being due to blood elements, namely, hematin in the tissue cells which would affect the percentage of reflection of red at 630 to 640 millimicrons and oxyhemoglobin at 580 and 540 millimicrons Such absorption bands occur uniformly throughout the curves obtained in the series reported here They are most evident in blonds and least evident in the Indians and negro, inasmuch as the superficial blood is more or less obscured by pigment The band at 630 millimicrons is probably the least constant, being the first to disappear with a decrease in the visibility of the exposed area The most constant region of change in reflection values is found at 580 millimicrons There is a sudden decrease in values in the curves of reflection from the yellow to the red regions, with a minimal value at about 580 millimicrons It is of interest that the dominant wavelength of the normal skin lies at about this point

Schultze (5), and Dorno (2) also noted this area of lowered reflection values in the general region of 540 to 580 millimicrons There was more variation in percentage of light reflected with changes in pigmentation in this spectral region than in the red end of the spectrum

The reflection of light in the violet end of the spectrum was uniformly low in percentage as compared with the red values but not as low as for the region 540 to 580 millimicrons Schultze noted that this corresponds to the energy distribution curves of Hausser and Vahle (4), and demonstrated radiation of total energy in the violet region of only 0 7 per cent of that radiated in the red region of the spectrum Other evidence in the literature in regard to the analysis of light reflected by the skin as affected by the presence of blood is singularly lacking

THE REFLECTION OF LIGHT FROM THE SKIN AS AFFECTED BY THE ABSENCE OF BLOOD

Probably the most striking of all color changes in the skin is that which appears after death We attempted a comparison of the reflection values and their analyses in the living subject with specimens obtained postmortem An area of skin about 6 by 6 cm was obtained from the lower part of the chest, in each case soon after death One

specimen was from a blond, the other from an American Indian   The
skin was denuded of fat and blood, spread on a board and stretched
to approximately its normal size and held by thumb tacks   The
surface was wiped dry and readings were made immediately by plac-
ing the specimen against the usual opening in the water-cooled cell
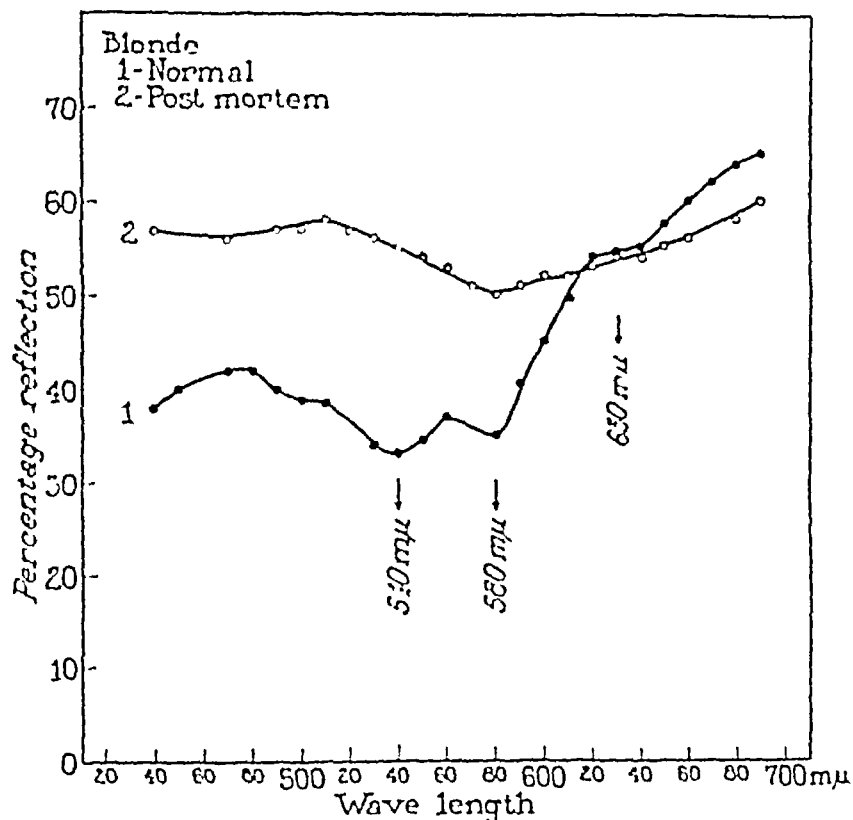
FIG 1 SPECTROPHOTOMETRIC REFLECTION CURVES 1, SKIN OF THE CHEST OF A
NORMAL LIVING BLOND, COMPARED WITH 2, SKIN OBTAINED POSTMORTEM
FROM THE CHEST OF A BLOND OF THE SAME TYPE

adapted to the color analyzer   For purposes of comparison, records
were obtained from a normal blond and a normal Indian of approxi-
mately the same body type and body area and of the same sex

Figures 1 and 2 represent spectrophotometric curves of the reflec-
tion values obtained in the normal subject as compared to the con-

ditions postmortem   Curve 1 in each case shows the relative differences in total light reflected by the skin of the blond type as contrasted
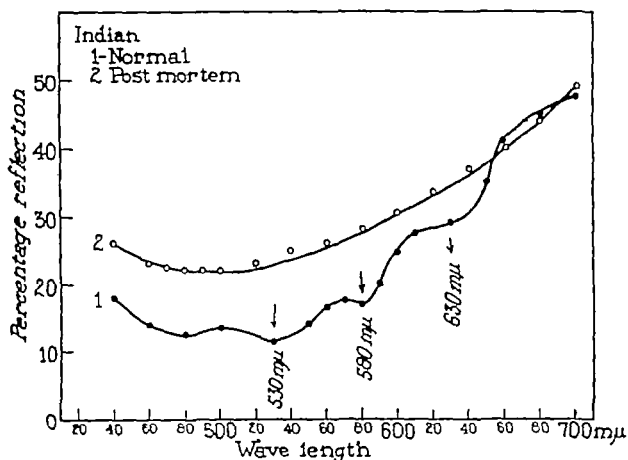
FIG 2 SPECTROPHOTOMETRIC REFLECTION CURVES *1*, SKIN OF THE CHEST OF A NORMAL LIVING OSAGE INDIAN, COMPARED WITH *2*, SKIN OBTAINED POST MORTEM FROM THE CHEST OF AN OSAGE INDIAN OF THE SAME TYPE

TABLE 1

Analyses from spectrophotometric curves of figures 1 and 2 with a comparison of living blond and Indian skins with specimens obtained postmortem

Type	Total color ex citation	Red	Green	Violet	Hue	Purity	Brilliance
	units	*per cent*	*per cent*	*per cent*	*milli microns*	*per cent*	*per cent*
Blond							
Living	7,339	38 8	34 9	26 3	586	27	40 0
Postmortem	9 664	36 3	36 3	27 4	575	23	51 4
Indian							
Living	3,015	44 8	32 9	22 3	595	39	16 7
Postmortem	4,895	39 7	37 2	23 1	580	40	27 0

to that of the dusky Indian   The reflection values of normal Indian skin reach a figure of 50 per cent in the red end of the spectrum

The irregularities in the normal curves and the absorption regions or spectral bands at 630, 580 and 540 millimicrons, respectively, are to be noted. These are conspicuous by their absence in the curves from
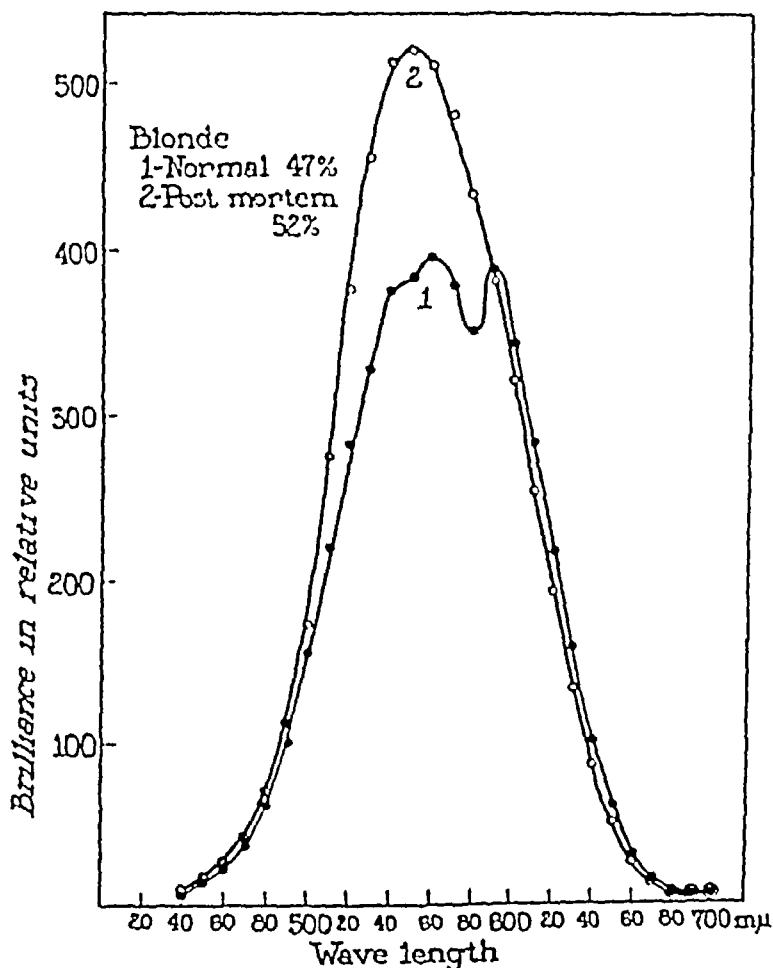
FIG 3 CURVES OF RELATIVE LUMINOSITY 1, NORMAL BLOND SKIN, AND 2, BLOND SKIN STUDIED POSTMORTEM, TO SHOW VARIATION IN BRILLIANCE

the bloodless skin obtained postmortem   Obliteration of these bands is probably the most striking demonstration possible of the relationship of these absorption regions to the blood elements present in normal skin   There is increased reflection of light in the region, 430 to

580 millimicrons   This is more marked in the case of the light skin than in the pigmented Indian skin, with little absolute change in the region of the orange and red   Analysis of these spectrophotometric curves into color excitation values and their conversion into the attributes of color are shown in table 1

The total excitation values reveal the extremes of light and dark types and are interpreted in terms of brilliance   The blood in the
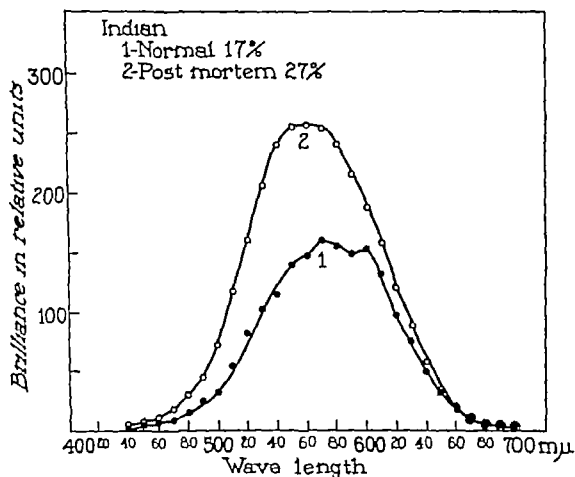
FIG 4 CURVES OF RELATIVE LUMINOSITY *1*, NORMAL SKIN OF OSAGE INDIAN, AND *2*, SKIN OBTAINED POSTMORTEM FROM AN OSAGE INDIAN OF THE SAME TYPE, TO SHOW VARIATION IN BRILLIANCE

superficial skin acts with the pigment to lower the percentage of light reflected, the blood itself accounting for 10 to 12 per cent of the degree of brilliance, as is seen in figures 3 and 4   The curves obtained from skin free from blood are symmetric and smooth, and those of the living subject tend toward diminished brilliance from wavelength 500 millimicrons toward wavelength 580 millimicrons, with a decided dip in the region of 580 millimicrons (oxyhemoglobin) which is more noticeable in the skin of the blond than in that of the Indian   Decided rearrange-

ment is effected in the distribution of the various percentages of red, green, and violet, as is shown graphically in figures 5 and 6   Curve 1 represents the normal linear arrangement of the summated values

FIG 5 PERCENTAGE COLOR EXCITATION VALUES 1, SKIN OF NORMAL LIVING
BLOOD, AND 2, BLOOD SKIN STUDIED POSTMORTEM

expressed in percentage form for both the blond and Indian living skins   Curve 2 shows the departure from the linear relationship obtained from an analysis of the same types of skin minus the blood only   The percentage decrease of light reflected in the red with corresponding elevation in the green end is to be noted   The violet is

affected least of all By plotting these values on the color triangle it will be seen that, although the percentage of purity or saturation remains unchanged in spite of the lack of blood elements, there is a

FIG 6 PERCENTAGE COLOR EXCITATION VALUES *1*, SKIN OF NORMAL LIVING OSAGE INDIAN AND *2*, SKIN OF OSAGE INDIAN STUDIED POSTMORTEM

shift in the hue or dominant wavelength from the spectral wavelength 586 millimicrons to 575 millimicrons in the blond and from wavelength 595 millimicrons to 580 millimicrons in the Indian

It has been demonstrated by a comparison of the living with bloodless skin that the superficial blood tends to alter the color of the skin

ment is effected in the distribution of the various percentages of red, green, and violet, as is shown graphically in figures 5 and 6   Curve 1 represents the normal linear arrangement of the summated values
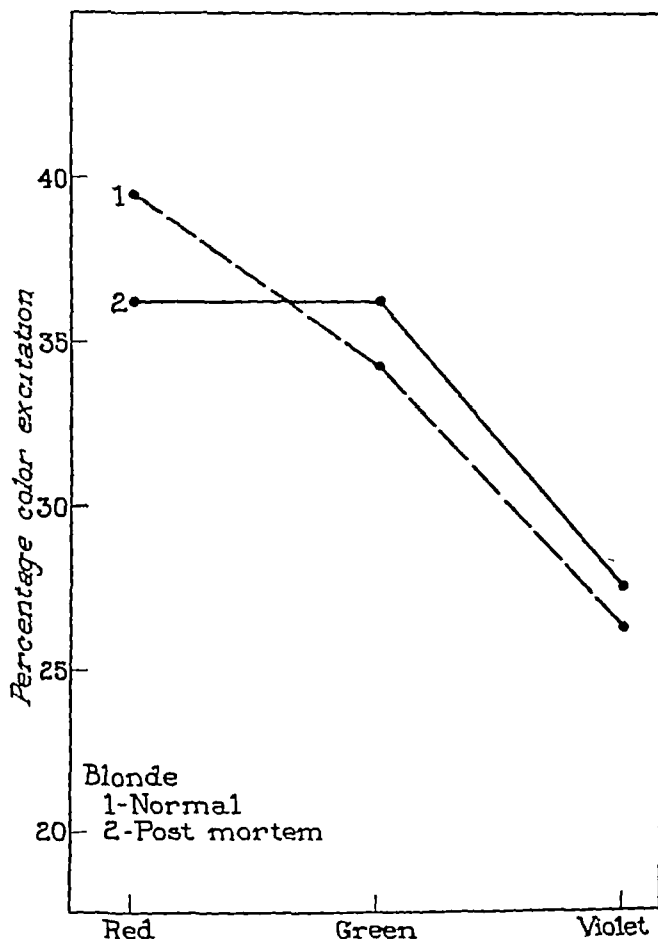
Fig 5 Percentage Color Excitation Values 1, Skin of Normal Living Blond, and 2, Blond Skin Studied Postmortem

expressed in percentage form for both the blond and Indian living skins   Curve 2 shows the departure from the linear relationship obtained from an analysis of the same types of skin minus the blood only   The percentage decrease of light reflected in the red with corresponding elevation in the green end is to be noted   The violet is

affected least of all   By plotting these values on the color triangle it will be seen that, although the percentage of purity or saturation remains unchanged in spite of the lack of blood elements, there is a

Fig 6 PERCENTAGE COLOR EXCITATION VALUES *1*, SKIN OF NORMAL LIVING OSAGE INDIAN, AND *2*, SKIN OF OSAGE INDIAN STUDIED POSTMORTEM

shift in the hue or dominant wavelength from the spectral wavelength 586 millimicrons to 575 millimicrons in the blond and from wavelength 595 millimicrons to 580 millimicrons in the Indian

It has been demonstrated by a comparison of the living with bloodless skin that the superficial blood tends to alter the color of the skin

by producing elevation in the hue, and a drop in the percentage of relative luminosity without affecting the percentage of purity to any extent

THE REFLECTION OF LIGHT AS AFFECTED BY THE SUPERFICIAL BLOOD WITH THE HAND PLACED IN THE DEPENDENT POSITION

Readings were made on the dorsum of the normal hand or the middle or ring finger, with the hand about 25 cm below heart level, using the
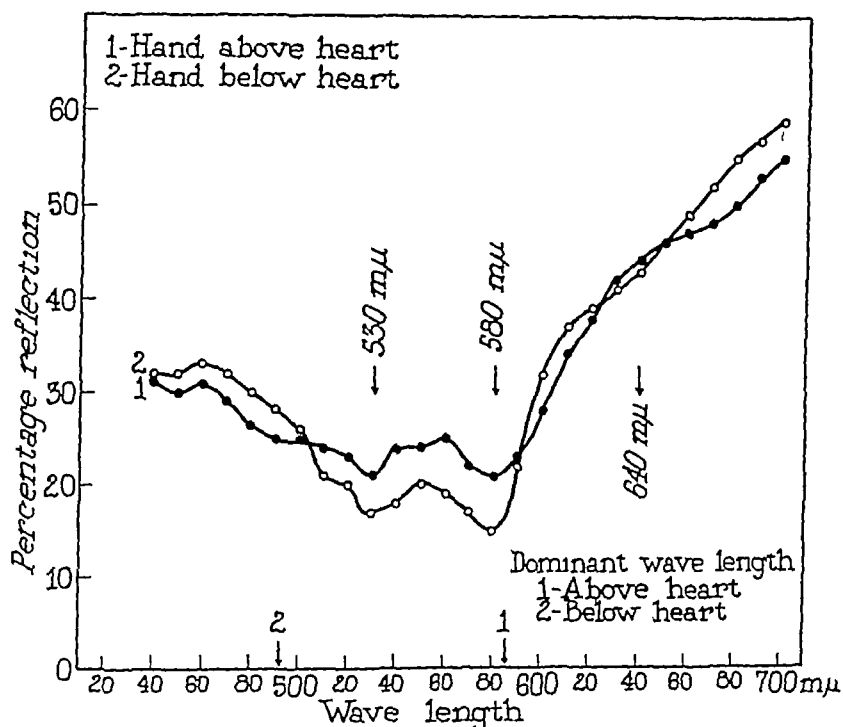
FIG 7 SPECTROPHOTOMETRIC REFLECTION CURVES OF SKIN OF HAND AS PLACED IN AN ELEVATED OR DEPENDENT POSITION 1, HAND 25 CM ABOVE HEART LEVEL, 2, HAND 25 CM BELOW HEART LEVEL

same areas of skin in each case   The change in color of the hand held in the dependent position depends to some extent on the prominence of the superficial venules, on the time the hand is allowed to remain in this position, and on the amount of interference with the return flow of blood   Cyanosis produced in this fashion has been shown by

Goldschmidt and Light (3) to be due to increased venous pressure peripherally and not related to the degree of oxygen unsaturation of the blood However, should any stasis develop to retard the flow of the blood, it is reasonable to suppose that the normal relation of carbon dioxide and oxygen in the blood and the tissues would be unbalanced and upset

Figure 7 shows two spectrophotometric reflection curves of the hand above and below heart level in a moderately brunet type The increased absorption effect produced in the region 530 milli-

TABLE 2

Analyses from spectrophotometric curves, illustrating the effect on the various components of color in the skin of placing the hand in the dependent position

Case	Type	Dominant wavelength		Purity		Relative luminosity	
		Above heart level	Below heart level	Above heart level	Below heart level	Above heart level	Below heart level
		milli- microns	milli- microns	per cent	per cent	per cent	per cent
1	Brunet (arsenismus)	590	592	45	44	21 7	23 6
2	Negro	585	590	50	33	7 5	10 9
3	Normal brunet	585	600	31	19	30 2	22 1
4	Normal blond	590	600	27	21	42 7	35 2
5	Japanese	587	605	31	8	24 3	18 2
6	Chinese	585	610	28	8	27 2	26 2
7	Normal blond	600	620	21	17	36 3	26 2
8	Marked brunet	592	492*	28	38	32 4	13 1
9	Normal brunet	586	493*	21	18	24 7	22 9

* Cyanosis

microns and 580 millimicrons when the hand is held below the heart is to be noted This plays a part in the change in the relations of color factors as determined by analysis of the reflection values in terms of excitation units, as previously described

Table 2 gives a list of records obtained in a similar manner from a variety of patients The reflection curves in case 9 are plotted in figure 7

Table 2 illustrates the variations in dominant wavelength, purity, and relative luminosity brought about by changing of the position of the hand from 25 cm above the heart to a point 25 cm below heart

level   Examples of the most common types of subjects are included
With the hand relatively devoid of superficial blood (above the heart),
the dominant wavelength is maintained in the spectral region 590
millimicrons, which corresponds to the region of pure sodium yellow

FIG 8 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC
DATA *1*, HAND ABOVE HEART, *2*, HAND BELOW HEART, AND *3*,
NORMAL CHEEK

If the hand is lowered to a point below heart level, the superficial blood
becomes more or less evident and its effect on the color of the skin can
be calculated
    In the heavily pigmented subjects (cases 1 and 2), an appreciable
change in hue (dominant wavelength) did not take place   Cases

3, 4, 5, 6 and 7 show a shifting toward the red, in one case (case 7) to a dominant wavelength 620 millimicrons   In this case, the normal hue had been established at 600 millimicrons, which may account for the marked rise above the normal variation

The relation of the color excitation values summated for red, green, and violet in percentage form for case 9 is shown in figure 8   This figure is an analysis of the spectrophotometric reflection curves of figure 7

Cases 8 and 9 exhibited a change in the dominant wavelength from the normal, 592 to 586 millimicrons, to the blue region at 492 millimicrons   This indicates cyanosis, irrespective of whether or not it is due solely to increased peripheral venous pressure or to changes in the oxygen unsaturation of the blood   Sheard (6), and Sheard and Brown (7) presented similar curves of cyanosis for cases of polycythemia vera following treatment with phenylhydrazine, with shifting of the dominant wavelength to the region 500 millimicrons   Cases 8 and 9 in table 2 show that such a condition is not rare even in normal subjects   The values obtained in each case are directly related to the surface area of superficial blood which attains visibility at any given time   This may occur with a good deal of facility in cases of polycythemia vera and becomes more evident clinically, inasmuch as the entire body is usually involved

Spectrophotometric and spectroscopic studies of the blood in experimental animals show characteristic absorption bands for hemoglobin in various degrees of oxygen saturation   In viewing the blood coursing through a rabbit's ear, Sonne (8) and others have been able to demonstrate the bands of oxyhemoglobin under normal conditions   Whenever the ear was pinched or the flow of blood was impeded in other ways, the presence of reduced hemoglobin was manifest.

Distention of the superficial venules with blood by means of gravity results, in most instances, in lowering the degree of hue   In other words, the color of the skin possesses a lower percentage of purity or saturation   This is least evident in cases in which the slightest variation in the relative percentages of red, green, and violet are excited, and it is most evident in the two cases of Oriental subjects in table 2 (cases 5 and 6)   In these cases the color of the skin approached very nearly an ashen gray, as exemplified by the drop in the purity or

level   Examples of the most common types of subjects are included
With the hand relatively devoid of superficial blood (above the heart),
the dominant wavelength is maintained in the spectral region 590
millimicrons, which corresponds to the region of pure sodium yellow

FIG 8 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC
DATA *1*, HAND ABOVE HEART, *2*, HAND BELOW HEART, AND *3*,
NORMAL CHEEK

If the hand is lowered to a point below heart level, the superficial blood
becomes more or less evident and its effect on the color of the skin can
be calculated

In the heavily pigmented subjects (cases 1 and 2), an appreciable
change in hue (dominant wavelength) did not take place   Cases

tion curves were obtained from a number of areas of the body which were analyzed into color excitation values and interpreted in terms of the three attributes of color (table 3)
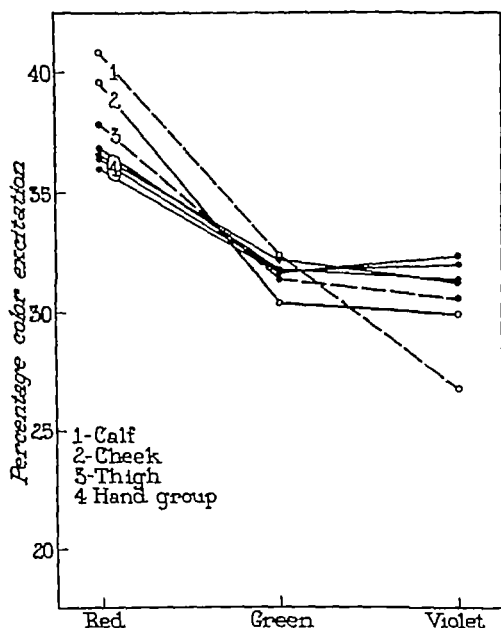
FIG 9 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC DATA FROM A CASE OF DIFFUSE ERYTHRODERMA

Readings made over various areas of the body and with the hand in an elevated and dependent position

The dominant wavelength was unquestionably in the red, being the highest over the malar prominence   In this region it had a value of 660 millimicrons   The hand, when emptied of venous blood by elevation, consistently showed a value of 640 millimicrons   This changed to a cyanotic hue when the hand was held in the dependent position   as was shown by the shift from 640 to 493 millimicrons.   In this condition the superficial venules filled with blood   The percentage values of

saturation to a level of 8 per cent   In only one case was there an increase in the purity (case 8) and this was associated with marked cyanosis

The relative luminosity suffers through the presence of superficial blood in proportion to the amount of pigment originally present to mask the effect   The blond subjects manifested the greatest reduction, the negro manifested very little reduction   When cyanosis was induced in one case (case 8) there was a drop in the total brilliance of almost 20 per cent

TABLE 3

Analyses of spectrophotometric curves of the skin into the fundamental attributes of color in a case of malignant erythroderma

Area	Dominant wavelength	Purity	Brilliance
	millimicrons	*per cent*	*per cent*
Cheek	660	12	26
Hand			
Above heart level	640	13	24
Below heart level	493	11	20
Thigh	630	10	28
Calf	600	22	24

From a study of the data, it would seem that cyanosis is an end stage of the accumulation of blood in the surface venules, when the flow becomes sluggish through the obstruction introduced by the load of gravity on the column of blood supported   An intermediary stage, or erythrosis, is noted which is similar to a phase of polycythemia vera   It is dependent on the surface area of the blood exposed for each centimeter of surface area and is more or less dependent on the velocity of flow through the part

THE REFLECTION OF LIGHT AS MODIFIED BY CERTAIN DISEASES OF THE SKIN

A few preliminary studies were made of dermatosis in which erythema from peripheral vasodilation was evidently present   Variations from the normal were noted in particular in the following cases

Case 1   The patient was aged forty years   The diagnosis was malignant erythroderma (diffuse universal erythema with branny desquammation)   Reflec-

Curves 2 and 3 (fig 9) and the group indicated by 4 represent a progressive increase in the percentage of violet until a definite break occurs in the linear relationship When the percentage of violet excited exceeds the percentage of green there is a shift in the hue from red to blue, which is manifested chiefly in the presence of cyanosis This was found to be characteristic of the cases of Raynaud's disease and of polycythemia vera following the administration of phenol hydrazine in the report of Sheard and Brown (7)

Case 2 The patient was aged forty-eight years The diagnosis was psoriasis vulgaris Experiments were carried out, with the hand at heart level, on a patch of psoriasis on the calf and an area of skin adjoining the patch The patient was placed in the prone position, to neutralize the effect of gravity, when the records from the leg were taken Figure 10 gives spectrophotometric curves of reflection from the three areas which are plotted for purposes of comparison There is similarity in all the curves, inasmuch as they reveal the decreased percentages of reflection at the regions 530, 580, and 630 millimicrons, respectively The curve

TABLE 4

Analyses of spectrophotometric curves of skin from normal areas and an area of psoriasis

Area	Dominant wavelength	Purity	Brilliance
	millimicrons	*per cent*	*per cent*
Normal skin of hand at heart level	598	19	21 0
Normal skin of calf	582	32	43 3
Psoriatic area of calf	620	23	22 8

from the surface of the patch of psoriasis shows an absorption zone at wavelength 580 millimicrons This area of skin was denuded of scales so that to the eye the patch was no longer the silvery white so characteristic of psoriasis but possessed the dull red color seen in large plaques over the glabrous surfaces and the trunk The records obtained from the normal skin of the hand and of the calf were similar except for the difference in the amount of reflection. This is interpreted in the subsequent analysis in terms of brilliance The hand, being darker than the calf, reflects less light

Analysis of these spectrophotometric curves into percentage color excitation values is shown in figure 11 and the results obtained after plotting these percentages are expressed in terms of dominant wavelength, purity and brilliance, as shown in table 4

The values of the dominant wavelength in this case (for the areas of normal skin studied) fell within that which may be termed the low to high normal, that is, from 582 to 598 millimicrons The hand held at heart level undoubtedly gave a higher reading than when held in an elevated position The patch of psoriasis was of a reddish hue clinically This was confirmed spectrophotometrically, since the dominant wavelength proved to be at 620 millimicrons or definitely in

the red   The difference in the readings for the three areas may be attributable to the influence of superficial blood in varying amounts, it was least in the velvet-appearing, soft skin of the calf in the prone position, and greatest in the psoriatic patch where the dilated capillaries attain visibility by virtue of the relatively
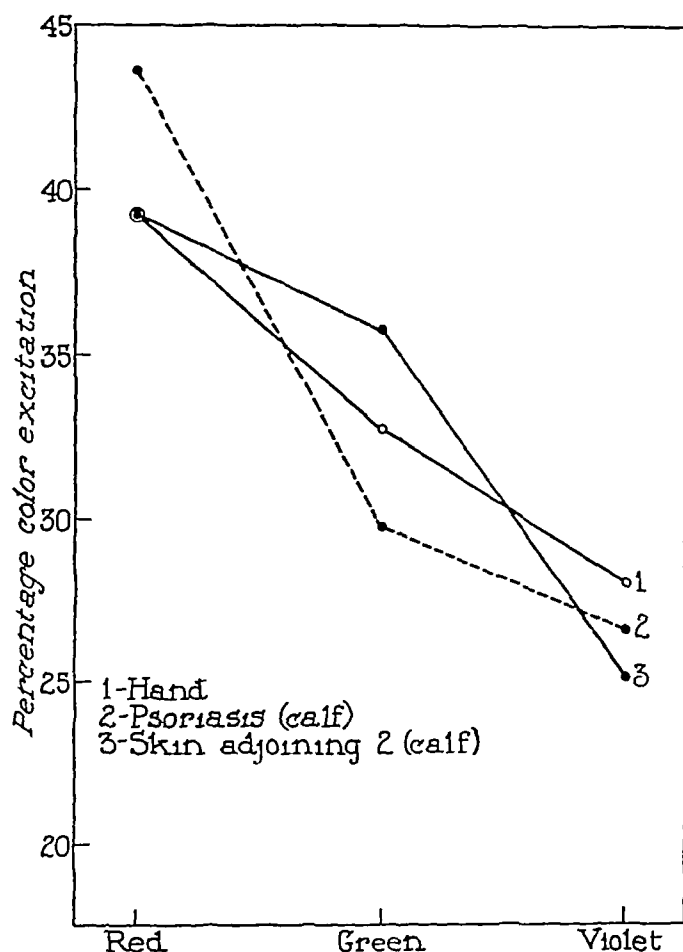
FIG 11 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC DATA OF CURVES IN FIGURE 10, *1*, HAND AT HEART LEVEL, *2*, PSORIATIC PATCH ON CALF, AND *3*, SKIN ADJOINING PATCH ON CALF

thin protective covering   This is a verification of the clinical sight of "bleeding points" following removal of the thin mica-like scales in psoriasis
    The relations of the percentages of red, green and violet color excited in each instance are best shown by figure 11   The normal linear distribution is preserved in curve 1 obtained from the record of the hand   Curves 2 and 3 differ in the rela-

tive percentages of red and green, thus altering the slope or the angle of the line plotted, and indicating the paucity of blood in the normal skin of the calf to the engorgement noted over the psoriatic patch

The analysis revealed a difference in percentage in purity in favor of the skin of the calf   This area also proved to be lighter in that there was elevation of the brilliance by 20 per cent, as was expected.   The patch of psoriasis was found to be of redder hue and of lower purity and brilliance than the normal skin adjacent to it

Case 3   The patient was aged thirty-eight years   The diagnosis was tuberculosis cutis (Sarcoid of Boeck)   This patient presented, among other features, areas of diffuse induration of the skin limited to the angles of the jaw and parotid region.   These were colored blue, apparently due to the presence of large quantities of venous blood in the dilated superficial blood spaces   Records were made

TABLE 5

Analyses of spectrophotometric curves of skin from normal areas and in areas of tuberculosis cutis

Area	Total color ex citation	Red	Green	Violet	Hue	Purity	Brilliance
	units	*per cent*	*per cent*	*per cent*	*milli microns*	*per cent*	*per cent*
Normal area on cheek	7,077	38 6	31 8	29 6	610	13	36 7
Lesion on right cheek	3,683	38 5	30 1	31 4	494*	19	18 5
Lesion on left cheek	3 788	36 9	30 6	32 5	495*	16	18 9
Hand elevated	5,040	38 1	32 2	29 7	610	12	26 3
Hand dependent	3,581	36 7	30 9	32 4	494*	15	17 0

* Cyanosis.

from each of these areas and also from the normal appearing skin over the malar prominence, as well as from the dorsum of the hand in the elevated and dependent positions   Analyses of the spectrophotometric data in terms of the various components of color are tabulated in table 5

Comparison of the values obtained from the various areas demonstrated that the colors of the elevated hand and the cheek were identical except for a difference in brilliance of 10 per cent   The dominant wave length at 610 millimicrons was a little nearer the red end of the visible spectrum than is usual for the normal subject, although this would be more likely to be true for the cheek than for the hand   The presence of superficial venous blood was manifested by a lowering in the amount of light reflected (decreased brilliance) and rearrangement of the percentages of red, green, and violet, with a shift in the dominant wavelength from the region of the red to the shorter (blue) spectral value of 494 millimicrons   This is consistently true for the areas in which the disease is found.   The presence of the superficial blood tends to diminish the brilliance.   The skin of the hand in the

dependent position and the skin over the two areas of lesions on the cheeks gave practically identical spectrophotometric data   This proves beyond doubt that they are of the same color

SUMMARY

Quantitative comparisons have been presented showing the variations in the color of the skin in health and disease as dependent on the content of superficial visible blood   Observations on specimens of skin from a blond subject and an Osage Indian obtained postmortem have been compared with records made during life in the same type of subjects to note the effect of the presence or absence of blood on the color of the skin   Furthermore, the effect of a change in the distribution of the superficial blood was observed by placing the hand in an elevated and dependent position in relation to the heart   Various types of dermatosis have been examined spectrophotometrically in order to demonstrate the effect of changes in amount and distribution of the superficial blood on the color of the skin

CONCLUSIONS

1 The blood in the superficial capillaries exerts a major influence on the color of the skin

2 Spectrophotometric observations of various areas of the human skin demonstrate considerable variation in the amount, quality and distribution of peripheral blood   Diseases of the vascular tree or the blood itself tend to accentuate these changes in the color of the skin

3 The pigment (melanin), in proportion to its density, acts as a screen to prevent the superficial blood from attaining visibility

4 Superficial blood plays a part in the curve of reflection of light from the surface of the skin and is evidenced by absorption zones at the spectral regions, 540, 580 and 630 millimicrons   The absorption band at 630 millimicrons is the least constant, whereas the others persist in all records except in those obtained from specimens of skin devoid of blood (postmortem)

5 An abundance of oxygenated blood near the surface of the skin tends to shift the dominant wavelength from the region 590 millimicrons to the orange or red end of the spectrum, reaching a value as high as 620 to 660 millimicrons   There is a diminution of the purity or degree of hue and a lowering of the relative luminosity or brilliance

6 An abundance of venous blood tends to shift the dominant length toward the blue region of the spectrum (490 to 500 millimi indicating a condition of cyanosis   This is produced by an alt in the relative percentage values for red, green and violet   No they maintain a linear relationship, but the value for violet ϵ the value for green in conditions of marked cyanosis

7 Spectrophotometric records and analyses may assist in t lineation of changes due to combined alterations in the cont blood and pigment in the skin of human beings

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STUDIES ON RED BLOOD CELL DIAMETER

III The Relative Diameter of Immature (Reticulocytes) and Adult Red Blood Cells in Health and Anemia, Especially in Pernicious Anemia

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It has been shown (1) (2) (4) that curves of the frequency of the diameters of normal erythrocytes are symmetrical and uniform   In most cases of anemia classed as secondary, frequency curves of measurement of the red blood cell diameters are displaced to the left, or small side, of the normal and the mean diameter of the cells is less than normal   In contrast to this, in pernicious anemia in relapse the frequency curve usually is displaced to the right and the mean diameter is greater than normal   In marked remissions of pernicious anemia induced by the feeding of suitable amounts of liver or potent liver extracts diameter frequency curves often approach normal (3) (9)

The irregularities of the frequency curves of the diameters of erythrocytes from pernicious anemia has aroused interest and Price-Jones (5) has suggested that in view of the apparent skewness of these curves there may be three elements of red blood cell formation in the bone marrow, the normal, giving the basic curve, together with the smaller cells (anemic) and larger (pernicious) cells which impart the skewness   It was suggested to me by Dr George R Minot that a study of the relative diameter of the young blood corpuscles (reticulated red blood cells, reticulocytes) to adult red blood cells might throw light on the nature of the skewness and that such observations upon normal red blood cells and those from various types of anemia might give information concerning blood formation   Heretofore no especial attention appears to have been given to the actual or compara-

tive sizes of the reticulocytes under varying conditions   Hawes (6) in 1909, described them as uniformly larger than non-reticulated cells Minot and Lee (10) and Key (7) have noted the occurrence of small reticulocytes   Gram (8) writes that "vital-stainable corpuscles" are "relatively thick elements" and "may dominate the picture in periods of forced regeneration of simple anemias and in hemolytic jaundice " Knowledge concerning the growth of tissue cells has been obtained by determinations of the nuclear-cytoplasmic ratio and this favored the idea that a comparison of the size of the mature and immature erythrocytes might help to explain certain features of the formation of red blood corpuscles   Twenty-six observations of this sort have been made of the erythrocytes of 5 normal and 19 pathological bloods, particularly from cases of pernicious anemia, and this paper concerns the results obtained

METHOD

Measurements of the red blood cell diameters were made from some specimens of capillary and some of venous blood, stained supravitally with brilliant cresyl blue between two thin glass coverslips   The blood was then stained with Wright's stain and the preparations mounted   The method of measuring the diameters was the same as that described in previous papers of this series (2) (3)   To obtain adult red blood cell diameters 250 consecutive cells having distinct boundaries were measured   Adult red blood cells were considered to be those with no intracellular structures such as reticulum, Howell-Jolly bodies, Cabot ring bodies or refractile granules (Isaacs)   The large, indistinct, pale red blood cells occurring in occasional fields of pernicious anemia blood were also omitted on the ground that they probably represent changes occurring after the cells are formed rather than abnormalities in blood formation   They are so rare that their inclusion would have changed nothing of importance in the data except the upper limit of red blood cell size   The occurrence of polychromatophilia may be taken to indicate improper staining by the method used   Hence to be as certain as possible that all reticulocytes were stained, no preparation containing polychromatophilic cells without reticulum was used for measurements   The 100 reticulocytes measured were found among or close to the adult red blood cells which had

been measured, so that, irrespective of absolute values, the relative values for adult red blood cell and reticulocyte diameters should be of significance

The absolute variations in the diameters of the red blood cells from venous and capillary blood were found to be very slight and no greater than those of the red blood cells from two different preparations of the same capillary blood taken at the same time   Furthermore, the distribution of red blood cell diameters and the relations of reticulocyte to adult red blood cell diameters were practically identical in the two types of blood

The mean or average diameter was computed arithmetically from the original data   The data were plotted for study as frequency curves and also as summation frequency curves on Whipple's arithmetic probability paper (see Fig 2) which converts a frequency curve showing perfect adherence to the "law of probability" into a straight line   The numerical ratio between mean reticulocyte and mean adult red blood cell diameter was computed for each case and is included in the data presented

COMPARISON OF RETICULOCYTES AND ADULT RED BLOOD CELLS

Figure 1 demonstrates by frequency curves the size relationships found to exist between the diameters of adult red blood cells and reticulocytes in normal bloods and in a case of pernicious anemia in relapse   A comparison of the curve for the percentage distribution of the diameters of 1250 adult red blood cells from five normal bloods with that for 500 reticulocytes from the same bloods indicates that the distribution of sizes is strikingly similar, but that the reticulocytes in normal bloods are uniformly somewhat larger than the adult red blood cells   Since normal bloods contain only about one per cent of reticulocytes this curve for adult red blood cells very nearly corresponds with comparable ones for all red blood cells of normal blood which have been published (1) (2) (4) (8)

The case of pernicious anemia recorded as case 18, table 1, for which data are charted in figure 1 had a red blood cell count of 1 8 million per cu mm   and a typical blood picture of that disease   The measurements are similar in all respects to those of the cells from the other nine pernicious anemia patients studied with low red blood cell

counts   The frequency curve (fig 1) for the diameters of the 250 adult red blood cells measured is similar to other diameter frequency curves which have been published (2) (3) (4) (5) for all cells in com-
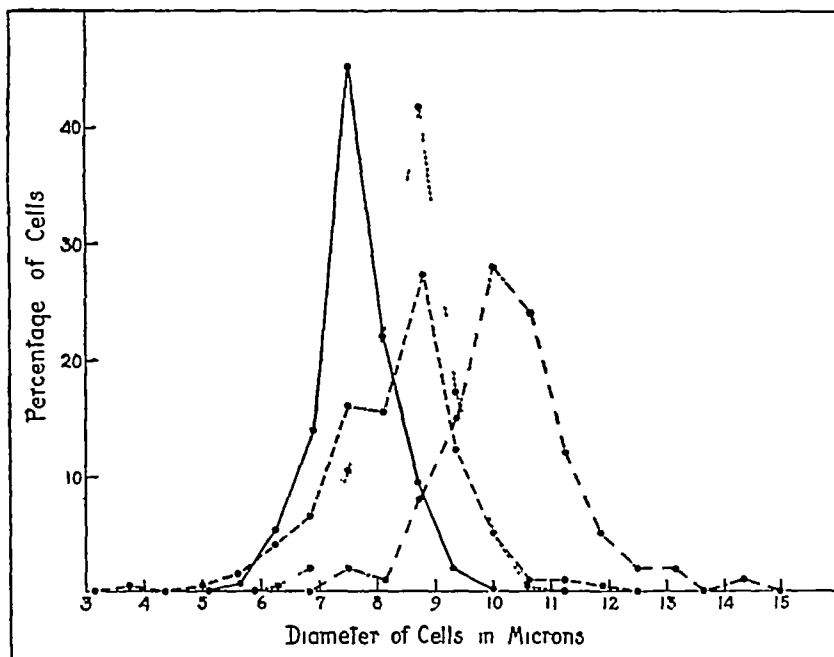
FIG 1 FREQUENCY CURVES OF THE SIZE RELATIONSHIPS BETWEEN THE DIAMETERS OF RETICULOCYTES AND ADULT RED BLOOD CELLS IN NORMAL BLOODS AND IN A CASE OF PERNICIOUS ANEMIA

The curves for normal bloods are constructed from the measurements of 1250 adult red blood cells and 500 reticulocytes from 5 individuals   The curves for the cells from a case (no 18, table 1) of pernicious anemia are constructed from the measurement of 250 adult red blood cells and 100 reticulocytes

——————— Normal adult red blood cells

——————— Normal reticulocytes

— —— —— Pernicious anemia adult red blood cells

— — — Pernicious anemia reticulocytes

parable cases of pernicious anemia   A comparison of this curve with that for 100 reticulocytes from the same blood shows a similarity of shape which, allowing for the smaller number of cells measured, is

quite as striking as that between the diameter frequency curves for reticulocytes and adult red blood cells from normal bloods

Table 1 shows the results of measurements of adult red blood cells and reticulocytes from 24 persons   These may be grouped in three classes, normal (5) "secondary" anemia due to various causes, (10) and pernicious anemia (9)

A   Normal bloods

Cases 1 to 5 were from healthy individuals who had normal blood pictures and the figures for their adult red blood cells conform fairly well to those obtained by other investigators for normal red blood cells   It is recognized that the adult red blood cell size of case 3 is lower than that of any normal blood which has been reported in this series of papers   The specimen was from a young adult male on whom repeated blood examinations have been made during the past four years without any abnormalities having been detected.

Normal reticulocytes average about 1 micron larger in diameter than normal adult red blood cells   The average of the means is 8 6 microns for the reticulocytes as against 7 6 microns for the adult red blood cells   The ratio of mean reticulocyte diameter to mean adult red blood cell diameter varies from 1 12 to 1 15   The frequency curves obtained by plotting the averaged data from these 5 normal bloods is shown in figure 1   Summation-frequency curves of reticulocyte and adult red blood cell diameters from these bloods are so nearly straight that nothing but errors of measurement could be postulated to explain deviations and in each case the reticulocyte diameter distribution curve is almost parallel to that for adult red blood cell diameter

B   "Secondary" anemia

In the cases of "secondary" anemia the reticulocyte-adult red blood cell diameter ratio (table 1) is lower than in the normal individuals

The measurements marked 4b and 5b (table 1) were made on cells taken from normal individuals (cases 4 and 5) three days after the loss of from 500 to 600 cc. of blood, while measurements labelled 4a and 5a were made just before the blood was removed   In each case the red blood cells had fallen approximately half a million per cubic millimeter

TABLE 1

The diameter of adult and reticulated red blood cells

Normal blood

Case	Red blood cells (millions per cu mm)	Adult red blood cells (microns)						Reticulocytes (microns)					Ratio* Reticulocytes/Adult
		Lower size	Upper size	Spread	Dispersion	Median diameter	Mean diameter	Lower size	Upper size	Spread	Mean diameter	Difference of means	
1	4.7	5.0	9.4	4.4	1.1	8.1	7.8	6.9	10.6	3.7	8.8	1.0	1.12
2	4.9	6.3	10.0	3.7	1.0	7.9	7.7	6.9	10.0	3.1	8.7	1.0	1.12
3	4.9	6.3	8.8	2.5	1.2	7.4	7.0	6.3	9.4	3.1	8.0	1.0	1.14
4a	5.0	6.3	9.4	3.1	1.1	8.0	7.7	7.5	10.0	2.5	8.8	1.1	1.15
4b	4.5	6.3	10.0	3.7			7.9	7.5	10.6	3.1	8.7	0.8	1.10
5a	4.8	6.3	10.0	3.7	1.2	8.0	7.8	8.1	11.3	3.2	9.0	1.2	1.15
5b	4.3	6.3	10.0	3.7			7.8	7.5	11.3	3.8	8.9	1.1	1.13

"Secondary" anemias

Case	Red blood cells (millions per cu mm)	Reticulocytes (per cent)	Adult red blood cells (microns)				Reticulocytes (microns)					Ratio* Reticulocytes/Adult	Causes of anemia
			Lower size	Upper size	Spread	Mean diameter	Lower size	Upper size	Spread	Mean diameter	Difference of means		
6	4.3	0.6	5.6	9.4	3.8	7.5	6.9	9.4	2.5	8.2	0.7	1.10	Hemorrhage, chronic
7	2.0	3.6	5.6	10.6	5.0	8.0	6.9	11.3	4.4	8.6	0.6	1.08	Hemorrhage, acute
8	2.8	7.0	5.6	10.6	5.0	7.7	6.3	10.6	4.3	8.3	0.6	1.07	Hemorrhage, acute
9	1.8	20.0	5.0	10.6	5.6	7.4	6.3	11.3	5.0	8.1	0.7	1.10	Repeated hemorrhage
10	3.2	17.0	4.6	9.9	5.3	6.7	5.0	10.0	5.0	7.3	0.6	1.09	Congenital hemolytic jaundice
11	3.5	12.5	5.0	9.4	4.4	7.0	6.3	9.4	3.1	7.6	0.6	1.09	Congenital hemolytic jaundice
12	2.2	5.0	5.0	10.0	5.0	7.2	6.3	10.6	4.3	8.0	0.8	1.10	Chronic myelogenous leukemia
13	3.2	21.0	4.4	10.6	6.2	8.0	6.9	10.6	3.7	8.4	0.4	1.06	Methemoglobinemia
14	1.6	15.5	5.0	9.4	4.4	7.2	5.6	10.0	4.4	7.6	0.4	1.06	Cancer
15	1.2	2.3	3.8	8.8	5.0	7.0	5.0	11.3	6.3	7.4	0.4	1.06	Cancer

Pernicious anemia (with low red blood cell counts)

Case	Red blood cells	Hemo-globin	Reticu-locytes	Adult red blood cells				Reticulocytes				Differ-ence of means	Ratio Reticulocytes Adult
				Lower size	Upper size	Spread	Mean diameter	Lower size	Upper size	Spread	Mean diameter		
	millions per cu. mm.	per cent	per cent	microns	microns	microns	microns	microns	microns	microns	microns	microns	
16	1 4	20	0 8	3 8	12 5	8 7	8 2	7 5	15 0	7 5	10 6	2 4	1 29
17	1 2	47	0 3	3 8	12 5	8 7	8 0	5 6	13 8	8 2	10 1	2 1	1 25
18	1 8	48	1 4	3 8	11 9	8 1	8 4	7 5	14 4	6 9	10 3	1 9	1 23
19	2 1	47	0 9	5 0	11 9	7 5	7 9	6 9	12 5	5 6	9 9	2 0	1 25
20	1 2	37	3 6	3 8	12 5	8 7	8 4	6 3	15 0	8 7	10 6	2 2	1 26
21	2 2	61	0 3	4 4	11 9	7 5	8 5	8 1	13 8	5 7	10 6	2 1	1 21
22	0 6	17	8 3	5 0	11 9	6 9	8 0	6 3	13 8	7 5	10 2	2 2	1 28
23	2 0	47	6 4	5 0	11 9	6 9	8 0	6 9	13 1	6 2	10 1	2 1	1 27
24	1 6	36	1 8	5 0	11 3	6 3	8 5	8 1	16 9	8 8	10 4	1 9	1 22

* The second decimal place is omitted in the table but was used to determine the ratio $\dfrac{\text{Reticulocytes}}{\text{Adult}}$

and about 4 per cent of reticulocytes were present indicating a response of the bone marrow   It is interesting that there is a slight increase in the adult red blood cell diameter in case 4 and no change for this value in case 5, while in both cases the reticulocytes are smaller than before the loss of blood so that the reticulocyte adult red blood cell diameter ratio dropped from 1 15 to 1 10 in case 4 and from 1 15 to 1 13 in case 5   A larger series of similar cases must be studied before the importance of these changes can be properly evaluated

The anemia in cases 6 to 9 was due to hemorrhage   Case 6 occurred in a woman who had had menstrual disturbances with abnormal blood loss and a slight anemia for several months before the specimen was taken   The mean adult red blood cell diameter while within normal limits, is smaller than that of most normal bloods, and the difference, 0 7 micron, between the mean reticulocyte and adult red blood cell diameters is definitely decreased, giving a reticulocyte-adult red blood cell diameter-ratio of 1 10   Cases 7 and 8 occurred in patients who had had a single severe hematemesis within a week of the time the specimen was taken   In these cases the mean adult red blood cell diameter is not abnormal, but the distribution has been changed, as is indicated by the increased value for the "spread "   The spread of the reticulocytes is also somewhat increased   Again the differences between the mean reticulocyte and adult red blood cell diameters are reduced, so that the ratios between them are 1 08 and 1 07, but again the response of the bone marrow was slight   Case 9 occurred in a patient who had had repeated severe hematemeses during the five weeks previous to the day the specimen was taken, and while the "spread" of the adult red blood cells is definitely increased the mean adult red blood cell diameter is quite small   The "spread" of the reticulocyte diameters is also increased and the mean reticulocyte diameter is correspondingly reduced so that the reticulocyte-adult red blood cell diameter ratio is 1 10, the same as that of the milder prolonged anemia due to blood loss (case 6)

Cases number 10 to 15 inclusive were of secondary anemia from causes other than hemorrhage   Case 10 and 11 were of congenital hemolytic jaundice and were both severe enough to justify splenectomy The measurements of the cells from these two bloods are strikingly similar and resemble those reported by Whitcher (11), both retic-

ulocyte and adult red blood cell "spreads" being increased and both sets of mean diameters being decreased considerably, so that the reticulocyte-adult red blood cell diameter ratio is 1 09 [1]

Case 12 was an example of "secondary" anemia accompanying chronic myelogenous leukemia and the measurements are very similar to those of case 9 with anemia from acute blood loss

Case 13 exhibited varying degrees of anemia associated with proved methemoglobinemia existant for about eighteen months   At the time the specimen was taken the patient's red blood cell count was increasing and was about 2 5 million per cubic millimeter   The measurements of adult red blood cell diameters fail to show decreased mean cell size, but the "spread" is markedly increased   The many reticulocytes (21 per cent) are relatively small and the ratio of the mean diameters of the reticulocytes and adult red blood cells is low (1 06)

Case 14 showed a severe anemia due to cancer   The blood showed a high reticulocyte count, nucleated red blood cells and much poikilocytosis   The measurements show small adult red blood cells and reticulocytes and the ratio between their sizes is reduced to 1 06

Case 15 was the most interesting single case of the series   A blond Norwegian of 53 had severe anemia of about three months' duration His history and physical examination suggested that he had pernicious anemia   He was transfused twice with blood and liver feeding commenced, but he died four days after the first transfusion   At autopsy the cause of death proved to be a scirrhous carcinoma of the stomach with metastases to the regional lymph nodes, liver and bone marrow   The measurements of the red blood cells after the first transfusion showed marked reduction in the mean adult red blood cell diameter and more than proportionate reduction in the mean reticulocyte diameter so that the ratio between them was reduced to 1 06   Until the time of death one of the chief findings which weighed against the diagnosis of pernicious anemia was these measurements of his red blood cell diameters

Frequency curves plotted from measurements of the diameters of adult red blood cells in "secondary" anemia are similar to those

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[1] An unpublished case of congenital hemolytic jaundice showing an increase in the mean diameter of the reticulocytes has been observed by Means and Thomas

obtained by other observers for all types of cells in similar bloods and the curves for reticulocyte diameters show corresponding shapes, but are not displaced as far to the right as are these curves for normal bloods   Summation-frequency curves on arithmetic probability paper are nearly straight for both reticulocyte and adult red blood cell diameters, if enough cells are measured to overcome the effect of the increased variation in size ("spread") found in "secondary" anemia bloods   Again the curve for reticulocyte diameter frequency is displaced to the right less than in normal bloods

C Pernicious anemia

Measurements from 9 cases of pernicious anemia with low red blood cell counts (cases 16–24, table 1) show that in every instance the mean red blood cell diameter is large, as is also the spread of both adult red blood cells and reticulocytes   The mean adult red blood cell diameter is close to normal in cases 19, 22 and 23, but the mean reticulocyte diameter is distinctly above normal   Since the mean reticulocyte diameter is relatively very large, the ratios of the mean diameters are from 1 21 to 1 29, or not less than 0 06 above the highest ratio obtained in a normal blood

Frequency curves of adult red blood cell diameter measurements from these bloods are similar to those reported for pernicious anemia bloods by other observers (2) (3) (4) (5), and the reticulocyte diameter frequency curves correspond in shape to those of adult red blood cells from the same bloods, but are displaced much farther to the right than are those from normal bloods

Summation-frequency curves plotted on arithmetic probability paper of 250 red blood cells from pernicious anemia bloods show deviations from the straight line plot for normal bloods, but in two cases in which 1000 cells of each were measured the resulting plots were as nearly straight as are corresponding curves from normal bloods

Figure 2 shows the summation diameter frequency curve obtained from the measurement of 1000 adult red blood cells from case 20, and a similar curve of diameter measurements of 100 reticulocytes from the same blood   These two curves are almost parallel and bear the same relation to each other that corresponding curves for red blood cells from normal bloods and secondary anemias do, except that the curve

for reticulocyte diameters is displaced farther to the right   This very striking difference between the sizes of reticulocytes and adult red blood cells, which is demonstrated both by the figures and the curves for each of the nine cases of pernicious anemia with a low blood cell count and which is much less marked in all the other cases studied may be characteristic of the blood picture of this disease
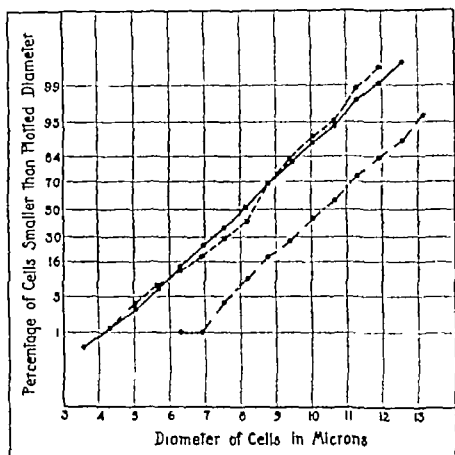
FIG 2 PERNICIOUS ANEMIA   SUMMATION DIAMETER FREQUENCY CURVES OF 1000 ADULT RED BLOOD CELLS 250 ADULT RED BLOOD CELLS AND 100 RETICULOCYTES FROM A CASE OF PERNICIOUS ANEMIA PLOTTED ON ARITHMETIC PROBABILITY PAPER

The curves for adult red blood cells show that the skewness of the curve based on only 250 cells disappears when a large number of cells (1000) are measured
————— 1000 adult red blood cells
— — — 250 adult red blood cells
— — — 100 reticulocytes

DISCUSSION

The striking parallelism between the adult red blood cell and reticulocyte diameter distribution curves furnishes further evidence that the formation of reticulocytes is most intimately connected with

the formation of adult red blood cells   In considering the relation of reticulocytes to adult red blood cells, there are two obvious possibilities   One is that the reticulocyte in the peripheral blood is a young red blood cell which will lose its reticulum in a few days, shrink somewhat in size, and become an adult cell   The other possibility is that reticulocytes are liberated from the marrow somewhat prematurely, and that they remain in the peripheral blood as such until dissolution takes place, but that the rate of their liberation is approximately proportionate to the rate of liberation of mature red blood cells   The present studies do not lead to deductions in favor of either of these hypotheses   The former is much the most probable one because one may observe extrusion and loss of reticular material in cells kept in plasma outside the body at 37 5°C   The investigation does indicate that a close correlation exists between the sizes of reticulocytes and adult red blood cells in normal and pathological bloods

The measurements from the twelve cases of "secondary" anemia recorded in table 1 indicate that when an extra load is thrown upon the bone marrow by "chronic" hemorrhage or red blood cell destruction it responds by throwing out reticulocytes which are relatively smaller than normal reticulocytes   (The actual measurements are smaller in only a fraction of the cases)   This is not what one would expect if the response of the bone marrow were simply one in which immature cells which would ordinarily remain in the marrow for a few days longer were liberated early, for studies of the bone marrow have shown that the red blood cells in it grow smaller as they mature

In the four cases (4b, 5b, 7 and 8) from which red blood cells obtained soon after hemorrhage were measured, it is reasonable to suppose that most of the adult red blood cells had been present before the hemorrhage occurred so that there should be no marked abnormality in their measurements   In these bloods, however, the reticulocytes are relatively small which shows that the mean reticulocyte diameters tend to be low, while the mean adult red blood cell diameters remain at normal values

In cases 6 and 9 one is justified in assuming that there had been time for some adjustment between the bone marrow and the circulating blood, or, in other words, that practically all of the adult red blood

cells present in the blood had been formed after the hemorrhages so that the parallelism between reticulocytes and adult red blood cells should be partially restored   That there was a trend in this direction is shown by the fact that the ratio between the mean diameters of reticulocytes and adult red blood cells is slightly higher than in the cases where there was not time for adjustment to take place   Although, under prolonged stimulus, the bone marrow was liberating smaller reticulocytes, the adult red blood cells were, by this time smaller also

From these and other data which have been published on the diameter of red blood cells in chronic "secondary" anemia it is apparent that the reaction of the bone marrow is to form red blood cells which are smaller than normal and which vary more in size as shown by the maximal and minimal measurements that yield the increased ' spread "   Jolly states that there is first a slight increase in mean diameter followed by the decrease observed in this study (12)

A comparison of the measurements of three types of bloods, normal, anemia from loss of blood in which there has been no time for readjustment, and anemia from blood loss in which some readjustment must have taken place, shows that there is a somewhat delayed parallelism between the diameters of reticulocytes and adult red blood cells   It would, necessarily follow that, in a patient who has had a single large hemorrhage, there will be a period in which the diameter ratio between reticulocytes and adult red blood cells would be less than that in normal bloods   Once the factor which stimulates the bone marrow to increased production is removed it should begin to form normal blood, including reticulocytes of normal size

Other observers have reported irregularities in summation-frequency curves of the diameters of red blood cells from cases of pernicious anemia (5) (2)   Measurements of 1000 red blood cells from each of two cases of pernicious anemia reported above resulted in curves which conformed to the "Law of Probability" as well as did curves for the diameter of red blood cells from normal bloods   Although the skewness of the curves obtained from measuring 250 red blood cells from the same cases was not marked, the result of measuring the larger number of red blood cells indicates that not all summation frequency curves of the diameters of red blood cells from pernicious anemia bloods are skew curves (see fig 2)

There is no obvious explanation for the apparently constant increase in the relative diameter of reticulocytes in cases of pernicious anemia having low red blood cell counts This increase is demonstrable in the diameter measurements, in the values for the ratio between mean reticulocyte and adult red blood cell diameters and is illustrated well by both frequency and summation-frequency curves plotted from the data It is interesting to note that in the two cases of anemia due to congenital hemolytic jaundice reported above the mean reticulocyte diameter was relatively decreased There is an excess of blood pigments in the plasma in this disorder as also occurs in pernicious anemia in relapse where the mean reticulocyte diameter is relatively increased In the congenital disorder the red blood cell diameter measurements were similar to those found in "secondary" anemia, with no increase pigments in the plasma, hence, it is not likely that the presence of pigment has any relation to the diameter of the red blood cells

CONCLUSIONS

1 There is a very intimate relationship between the sizes of reticulocytes (immature) and adult red blood cells in the same blood, as indicated by the fact that the shapes of the cell diameter distribution curves are almost identical

2 Reticulocytes of normal bloods average enough larger than adult red blood cells of the same bloods so that the ratio of mean reticulocyte diameter to mean adult red blood cell diameter varies from about 1 12 to 1 15

3 In chronic "secondary" anemias the reticulocytes are relatively smaller than those from normal bloods, so that this ratio varies from 1 06 to 1 10

4 In cases of pernicious anemia having low red blood cell counts (600,000 to 2,200,000 per cubic millimeter) the reticulocytes are much larger than the adult red blood cells, so that the ratio between the mean diameters is between 1 21 and 1 29 This may be characteristic of the blood picture of this disease

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STUDIES ON RED BLOOD CELL DIAMETER

IV THE DECREASE IN THE MEAN DIAMETER OF THE RETICULOCYTES AND ADULT RED BLOOD CELLS IN PERNICIOUS ANEMIA FOLLOWING LIVER THERAPY

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Minot and Murphy have shown (1) (2) (3) that in pernicious anemia in relapse one of the first signs of the regular response to adequate liver therapy is a temporary, marked increase of the reticulocytes that develops rapidly   After optimal amounts of liver are given the peak of the reticulocyte increase usually occurs between the sixth and tenth day seldom as soon as the fifth or after the tenth day   From that time they have definitely increased until they have returned to a similar level it is rarely less than seven or more than thirteen days, and usually eight to ten days (3)

Persons' studies have shown that in pernicious anemia with a low red blood cell count there is an increase in the mean reticulocyte (immature red blood cell) diameter, as well as in the mean adult red blood cell diameter (4)   In view of these findings several sets of measurements have been made of the immature and adult red blood cells from each of nine cases of pernicious anemia over periods ranging from the first twenty-one days to the first six months of remissions induced by the daily ingestion of large amounts of liver   In two of these cases frequent measurements were made during the rise and fall of the reticulocytes

The measurements on only 5 of the 9 cases are presented in table 1 to conserve space   The data, although obtained at irregular intervals on the other cases, are similar to those presented in graphic form in figure 1   The same method was employed to measure reticulocytes and adult red blood cells as that outlined in a previous paper (5)

TABLE 1

The blood of 5 cases of pernicious anemia treated with liver

Case number	Days	Red blood cells	Hemo-globin	Reticu locytes	Measurements of diameters in microns						Ratio † reticulocytes / adult
					Adult red blood cells				Reticulocytes		
					Spread	Disper sion	Median diam eter	Mean diam eter	Spread	Mean diam eter	
		millions per cu mm	per cent	per cent							
1	0*	1 4	20	0 8	8 7	3 4	8 7	8 2	7 5	10 6	1 29
	10	0 9	22	0 6	8 7	2 7	7 9	7 7	7 5	9 9	1 28
	16	1 7	29	15 5	9 3	3 0	8 2	7 9	5 6	9 1	1 14
	23	2 7	57	2 3	7 5	2 2	9 0	8 7	3 1	8 8	1 02
	32	3 6	72	0 2	8 1	1 9	8 7	8 3	4 3	8 7	1 04
	57	3 8	98	0 6	5 0	1 4	7 8	7 5	3 7	7 8	1 04
	126	5 0	106	0 3	5 0	1 5	8 2	7 9	3 7	9 4	1 18
2	0*	1 2	47	0 3	8 7	2 4	8 2	8 0	8 1	10 0	1 25
	7	1 4	49	2 0	8 1	3 0	8 1	7 7	6 2	10 0	1 30
	24	2 5	68	0 5	7 5	2 5	8 7	8 4	4 3	9 7	1 16
	56	3 5	94	0 3	5 6	1 7	8 0	7 7	4 3	8 8	1 14
	179	5 3	116	0 4	3 7	1 2	7 4	7 0	3 1	7 97	1 13
3	0	1 8	48	1 4	8 1	1 8	8 7	8 4	6 8	10 3	1 23
	12	1 6	35	13 1	6 8	2 7	7 8	7 0	5 0	8 9	1 26
	30	2 9	66	0 6	8 1	2 1	8 7	8 2	3 7	9 0	1 09
	95	4 7	82	0 2	5 0	1 4	8 3	8 0	3 7	8 2	1 02
	177	6 4	94	1 0	3 7	1 1	7 9	7 6	3 1	8 3	1 09
4	0*	2 1	47	0 9	7 5	2 2	8 1	7 8	5 6	9 8	1 25
	14	2 0	65	4 0	8 1	2 6	9 0	8 6	6 8	10 2	1 18
	18	3 0	62	6 4	8 1	2 0	8 5	8 3	4 3	8 7	1 05
	24	3 5	75	0 8	6 8	2 2	8 8	8 6	4 3	8 8	1 03
	50	5 1	110	0 05	6 8	1 6	7 2	6 7	3 7	7 6	1 13
	77	5 5	109	0 3	5 6	1 2	8 1	7 9	3 7	8 5	1 08
	166	5 7	110	0 2	4 3	1 2	7 8	7 6	3 7	8 5	1 12
5	0*	0 7		3 3	10 8	2 5	8 6	8 6	8 4	10 3	1 19
	3	0 8		2 9	9 6	3 1	8 7	8 5	8 4	10 8	1 26
	5	0 9		6 2	9 6	2 7	8 7	8 5	9 0	10 6	1 25
	6	0 9		12 0	9 6	3 3	8 3	8 1	9 0	10 6	1 32
	8			50 9	7 2	3 4	8 4	8 2	8 4	9 3	1 14
	10	1 4		36 4	9 0	2 4	8 7	8 6	6 0	9 2	1 07
	12			16 3	8 4	2 0	8 7	8 7	6 0	9 2	1 05
	19	2 4		4 1	7 8	1 8	8 5	8 6	4 2	8 7	1 01
	21	2 8		0 9	8 4	1 9	8 4	8 3	4 2	8 8	1 06

* Case 1 Liver started on 6th day   Case 2 Liver started on 4th day   Case 4 Liver started on 8th day   Case 5 Liver started on 2nd day

† The second decimal place is omitted in the table, but was used to determine the ratio $\frac{reticulocytes}{adult}$

Cases 1 to 4 inclusive were among the earliest cases treated by Minot and Murphy   They received only about 150 grams of cooked liver a day and hence their response was definitely slower than occurs in most cases given larger amounts of liver, kidney, or full doses of potent liver extract (3)

Changes in the Mean Diameter of Reticulocytes and Adult Red Cells following Liver Therapy in Pernicious Anemia
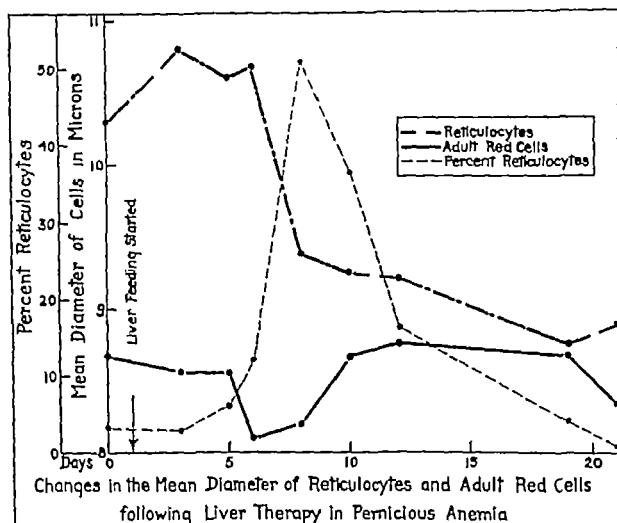
Fig 1 The Decrease of the Mean Diameter of the Reticulocytes and Adult Red Blood Cells in Relation to the Temporary Increase of Reticulocytes Produced by Liver Feeding in Pernicious Anemia

The curves in figure 1 which were constructed from the data presented for case 5 (table 1) are comparable to curves plotted for the other cases   The patient received daily 250 grams of raw liver pulp During the first five days of liver feeding there was a slight rise in the mean diameter of the reticulocytes, but no significant change in the diameter of the adult red blood cells   On the fifth day the rise in the reticulocytes began and reached a peak on the eighth day, gradually

falling to near normal by the nineteenth day From the fifth to the nineteenth day the most important changes in red blood cell size occurred They were (1) A marked drop in mean reticulocyte diameter from the sixth to the eighth day as the reticulocytes rose (2) A gradual decrease in mean reticulocyte diameter from the eighth day onwards (3) A distinct rapid drop in mean adult red blood cell diameter from the fifth to the sixth day followed by an increase from the eighth to the twelfth day to within 0 10 micron of the original size (4) A very gradual decrease in mean adult red blood cell diameter from the twelfth day onwards

In cases followed for longer periods of time the mean reticulocyte and adult red blood cell diameters decreased gradually so that normal proportions were usually found when the red blood cell count reached normal figures Not only did the actual diameter measurements become normal but the abnormally high ratio between the diameters of the reticulocytes and adult red blood cells approached normal, as the data in the last column of table 1 shows Both the spread (difference in diameter between the smallest and largest red blood cell found) and the dispersion (difference between the 16 per cent and 84 per cent grade taken from the curve of red blood cell size plotted on arithmetic probability paper) also approached normal as shown in table 1 Davidson and McCrie (7), using a different method to measure red blood cells, question the findings of Medears and Minot who state that "The mean diameter of the red blood cells in pernicious anemia may become normal in patients improved by a special diet rich in liver when the red blood cell count increases to between 4,200,000 and 6,300,000 per cubic millimeter" Our findings are in accord with the statement of Medears and Minot (6) It would appear that one of the signs to indicate that the patient has received and is receiving an adequate amount of anti-pernicious anemia substance is that the red blood cell diameters are normal or essentially normal

It is not likely that the initial rise in the mean diameter of the reticulocytes which occurred before the rise in the number of these cells is significant The pronounced drop in mean reticulocyte diameter and the less marked drop in mean adult red blood cell diameter, which occurred as the reticulocytes rose to a peak, are

outstanding rapid changes   The subsequent rise in mean adult red blood cell diameter coincident with the fall in the number of reticulocytes was detected in several cases   It is apparently a change of short duration and lasted only four days in case 5   It was an unexpected finding the cause of which is not evident.   The subsequent slow decrease in mean reticulocyte and adult red blood cell diameter, which continued until normal proportions were found, when the red blood cell count approached normal, adds further evidence to that in the literature that continued adequate liver therapy can permit the bone marrow in pernicious anemia to manufacture red blood cells of normal size

CONCLUSIONS

Measurements of the diameters were made of immature (reticulocytes) and adult red blood cells from 9 cases of pernicious anemia during treatment with liver

As the reticulocytes increased there occurred an initial drop in the mean adult red blood cell diameter which soon rose again almost to its original level   The size remained approximately the same while the red blood cells increased about 1 million per cubic millimeter and then progressively decreased to about normal as the red blood cell count rose above 4 5 million per cubic millimeter

The mean reticulocyte diameter decreased markedly as the reticulocytes rapidly rose   It then continued to decrease slowly becoming normal or essentially so when the red blood cells approached 5 million per cubic millimeter

Decrease to normal values in the mean reticulocyte and adult red blood cell diameters in pernicious anemia can result from continued adequate liver therapy causing changes in the bone marrow

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